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EMJ

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+ DIGIT-*ALL*: RARE DISEASES

+ EDITOR'S PICK

Tumour-Associated Hypoxia: Can We Give Chimeric Antigen Receptor T Cells More Breathing Space?

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"We guarantee that EMJ 5.3 will prove a thought-provoking read, no matter your expertise, and we hope it will serve as a catalyst for high-impact research"

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

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EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

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EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

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EMJ 5.3

Welcome

Dear Readers,

It is with great pleasure and pride that I welcome you to the newest issue of our multidisciplinary flagship journal. Bringing together expertise from various specialties to solve global challenges is now more important than ever, and this is reflected in the growing number of virtual, interdisciplinary conferences. Here at EMJ, it is our hope that with the contribution of this journal containing articles from various therapeutic areas, we will enable our readers to gain an insight into the newest developments across the medical field. We guarantee that *EMJ 5.3* will prove a thought-provoking read, no matter your expertise, and we hope it will serve as a catalyst for high-impact research.

EMJ 5.3 hosts a promising selection of content covering oncology, urology, haematology, rheumatology, respiratory, and reproductive health. In the field of oncology, we provide enthralling contributions such as the comprehensive review by Edechi et al. on breast cancer metastasis and the role of the epithelial–mesenchymal transition and immune system in the metastatic process. The Editor's Pick by Larios Martinez et al. discusses the proof of concept on utilising hypoxia to enhance precision targeting of CAR-T cell immunotherapy, a very important topic in the upcoming era of personalised medicine.

Following the theme of an increased need for individualised treatment is the review by Bitcon et al. on treatment options for interstitial cystitis/bladder pain syndrome, an enfeebling condition affecting 3% of the female population. If you are interested in reproductive health, Andreeva et al. have provided intriguing research results on the benefits of testosterone therapy in poor ovarian responders, the most challenging patients in reproductive health, undergoing *in vitro* fertilisation.

These articles are only the tip of the iceberg, so why not take this opportunity to broaden your scope of knowledge by reading all the fascinating articles that *EMJ 5.3* has to offer. In the following pages, you will also be treated to an interview with Dr Krishnan Ganapathy, president of the Apollo Telemedicine Networking Foundation (ATNF), and a topical feature by Baynam et al. on the application of digital health to rare diseases, highlighting the vast potential for the future. These are not to be missed!

Finally, I would like to thank all contributors for their valued collaboration and extend my appreciation to the entire EMG-Health team for their hard work during these unprecedented times. I hope you enjoy this eJournal, and remember that reading is essential for those who seek to rise above the ordinary.

Spencer Gore

Chief Executive Officer, EMG-Health

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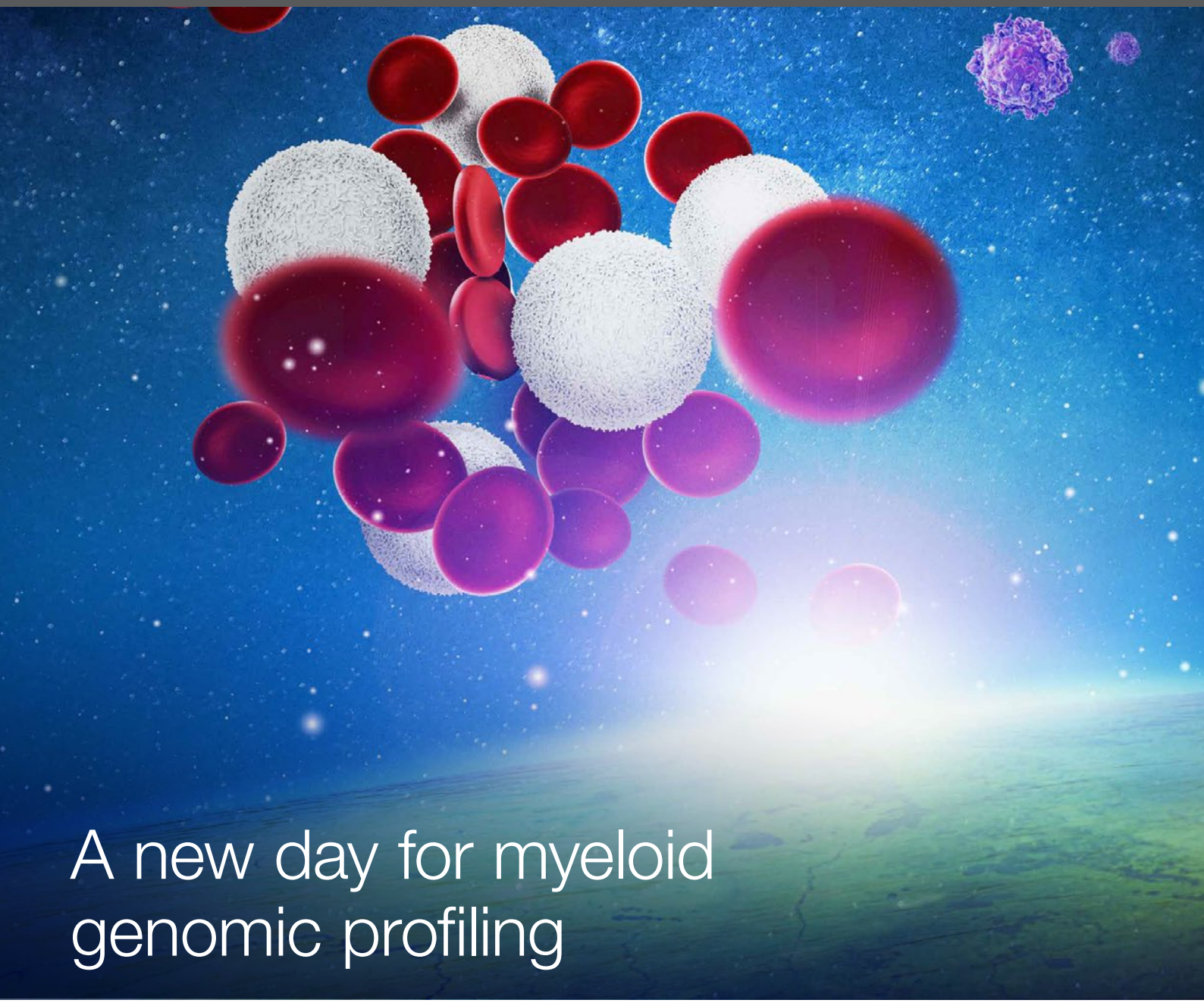

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Foreword

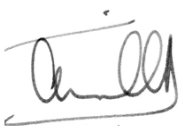
Dear Friends and Colleagues,

Welcome to this new issue of the *EMJ* flagship eJournal. Even while the COVID-19 pandemic is still ongoing, it is vital to progress with our regular professional, scientific, and educational activities. In this issue, I am more than happy to introduce to you a selection of interesting articles.

Three of the articles are in relation to oncology, my main expertise. In their article, Edechi et al. discuss the metastasis process of breast cancer, in particular the role of epithelial–mesenchymal transition, and the contribution of the immune system and potential role of immunotherapy. The paper by Larios Martinez et al. is of great interest in order to improve the efficiency of chimeric antigen receptor engineered T cells that are exclusively expressed or activated under conditions of profound hypoxia, a feature of several solid tumors, and is therefore my Editor's Pick for this issue. The last of the three papers, by Makhoul and Kieber-Emmons, puts into perspective the targeted cancer vaccine in order to overcome the antigenic heterogeneity in tumours as well as the heterogeneity of individual immune responses.

Interestingly, three of the other papers in this issue are in relation to immune function and autoimmune diseases. One of the papers, by Asghar et al., describes a case of Sjögren's syndrome complicated with Type-2 autoimmune hepatitis. The second reported a case of superficial ulcerating rheumatoid necrobiosis associated with methotrexate use in rheumatoid arthritis and, finally, Nobs dissected the tissue-specific immunity in the respiratory tract, a timely topic during the COVID-19 pandemic in view of severe respiratory syndrome seen with this infection, in which immune function and cytokines play major roles in the pathogenesis.

On behalf of the *EMJ* Editorial Board and myself, I wish you a pleasant read of this timely new issue of *EMJ*.



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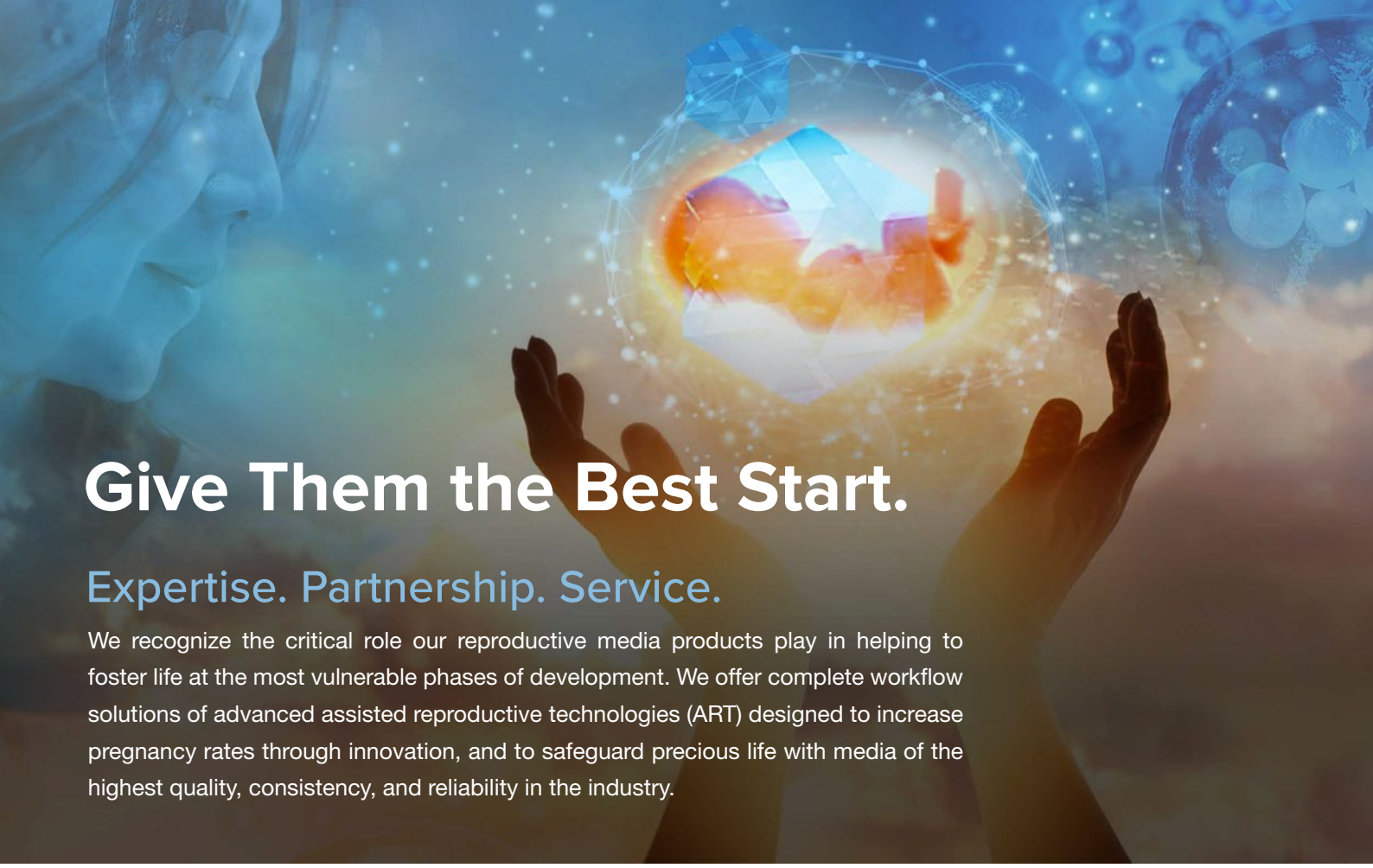

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Digit-*all*: Rare Diseases

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Abstract

Rare diseases are increasingly recognised as a global public health priority and contribute to significant and disproportionately high health system impacts. Accordingly, they present clinical and public health challenges, as well as opportunities for digital health solutions across the lifespan, including improved diagnosis, treatment, navigation and care coordination, and integration and coordination for broader societal and patient wellbeing. People living with rare diseases, individually and cumulatively, are digital disruptors. In this manuscript the authors describe some of the unique dynamics of the rare disease domain as they currently, or have the potential to in the future, apply to digital health; highlight some recent international rare diseases digital health initiatives; and touch upon implications for those with more common disorders.

INTRODUCTION

Rare diseases are increasingly recognised as a global public health priority.[1] Whilst rare diseases have a low prevalence individually, it is estimated that the combined prevalence is between 6 and 8% of the population,[2] equating to >400 million people globally. Most rare diseases have a genetic association, and are often severely debilitating, impair physical and mental abilities, and shorten life expectancy.[3] Rare diseases also contribute to significant and disproportionately high health system impacts, such as cost burden.[4] These characteristics present clinical and public health challenges, as well as opportunities for digital health solutions across the lifespan, including

improved diagnosis, treatment, navigation and care coordination, and integration and coordination for broader societal and patient wellbeing (e.g., linkage to education, disability, and community sectors). In this article, the authors describe some dynamics of the rare disease domain as they currently, or have the potential to in the future, apply to digital health; highlight some recent digital health initiatives in the international rare diseases domain; and touch upon implications for those with more common disorders.

SO, WHAT IS DIGITAL?

Some Useful Definitions

Digital is defined as recording or storing information as a series of the numbers 1 and 0, to show that a signal is present or absent; using or relating to digital signals and computer technology.[5]

"Digital health harvests data, information, and knowledge in real time from all societal activities, not just interactions with the health system and/or data traditionally regarded as 'health' data; uses sophisticated analytics to distil knowledge from these data; intervenes in the widest possible range of societal and economic activities and technologies to encourage and generate better health and better value for health investments."[6]

Digital health systems are not just about health and health system workflows, they are about life and life-flows. Comprehensive digital health systems extend beyond illness into other areas of the patient's life. That is, the flows extend across the breadth of human experience to include a person's health, family, education, (dis)ability, economic (dis)advantage, and the community to which they belong. They require real-world data, information, and knowledge in real time, from all societal activities, to generate better health value. They also require approaches that are person-centric, decentralised, or distributed, and empower healthcare providers to actively participate and partner with each other and their patients, not simply to control a fixed outcome.

WHY DIGITAL HEALTH AND RARE DISEASES?

In addition to the very significant patient needs, there are a number of other dynamics of the rare diseases domain that make it fecund for digital health advances and implementation.

Digital People

Notwithstanding the complexity, fundamental humanity, and multidimensionality of living with a rare disease, rare monogenetic (single gene) diseases may be as close to a causally binary chronic disorder that medicine offers. That is, the presence of a single causative factor, for instance a mutation in one's DNA, can invariably (complete penetrance) or often (incomplete penetrance) lead to a manifest disorder. Put another way, a disruption in a digital (DNA) code can invariably or often result in the presence (1) or absence (0) of a chronic and severe condition. People living with rare diseases can be thought of as living digital disruptors; their diseases can have a binary model and they can shift the fundamental expectations and behaviours in a culture, market, industry, technology, or process that is caused by, or expressed through, digital capabilities, channels, or assets.

Signal-to-Noise Ratio

Signal-to-noise ratio is a measure used in science and engineering that compares the level of a desired signal to the level of background noise. A signal is a meaningful input whereas noise is a meaningless or unwanted input. The severity of rare diseases provides a high signal that is primed for a (digital) readout. The high signal-to-noise ratios found in rare diseases are caused by the magnitude of both individual features of rare disorders and other unusual signatures of the condition itself that can be readily detected. These signals are also grounded in molecular biological pathways to provide insights into disease pathogenesis and its management. When combined, these factors provide clarity from the extremity of rare diseases. Similarly, the magnitude, and sometimes the relative speed of impact from an intervention, may be overt, which allows timely and effective monitoring. Accordingly, William Harvey (1578–1657), who was the first to describe the systemic circulation

of blood pumped to the brain and body by the heart, noted: "Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by the careful investigation of cases of rarer forms of disease. For it has been found in almost all things, that what they contain of useful or of applicable nature, is hardly perceived unless we are deprived of them, or they become deranged in some way."[7]

Multisystemic

Cumulatively, rare diseases affect all body systems, and individually they are often present with multisystem features. They traverse all medical specialities and the life-course. They are also exemplars for systems biology and the implementation of multi-omic approaches (i.e., combinations of genomics, epigenomics, phenomics etc). Because their individual rarity is combined with many layers of common elements, they require and are uniquely suited to cross-border, multi-health system care. Rare diseases are medically multisystemic, require systems biology, and health systems approaches that adapt to the flows of life.

Runs on the Board

The dynamics of rare diseases have already supported the implementation of digital health approaches in genomic healthcare, medical imaging and deep phenotyping, and international data sharing and matchmaking.[8]

Healing Hands

Caring for people with rare diseases requires combining traditional and physical approaches with modern technology. Put another way, there is a need to meld high-touch digital (the digits of healing hands) and high-tech digital (technology) approaches.

WHAT'S HAPPENING IN DIGITAL HEALTH FOR RARE DISEASES?

Increasingly, and particularly over the last 2 years, there has been global engagement and convergence in digital health for rare diseases. Some notable examples are touched upon below.

Rare Diseases International and the World Health Assembly

The 23rd May 2019 marked a historic day for the rare diseases movement, with rare diseases featuring on the agenda of the World Health Assembly (WHA) for the first time.[9] Rare Diseases International (RDI) contributed towards the formal and informal events that shone a spotlight on the importance of including rare diseases in universal health coverage in order to leave no one behind. At these events it was noted that rare diseases are at the forefront of digital health and they exemplify the high added value of regional and global approaches. They also showed that new digital tools are already being used to address the challenges experienced by the >400 million people living with a rare disease, enabling them to connect highly isolated patients, enable access to and acceleration of diagnosis, refer to specialised medical expertise, gather and share expertise on highly complex care, and advance clinical research.

A formal side event, part of the official WHA agenda and sponsored by permanent missions from the European Union, Romania, and Kuwait, used rare disease case studies to highlight the potential of digital health to achieve universal health coverage. The event was co-sponsored by an additional nine Member States, demonstrating the high level of interest and support.

The Global Commission to End the Diagnostic Odyssey for Children with a Rare Disease

The Commission is a global approach to accelerate time to diagnosis for children living with rare diseases.[9] It uses a multidisciplinary group of international experts to develop a road map to accelerate the time to diagnosis for children living with rare diseases. They also develop, deploy, harmonise, and interoperate digital tools developed through pilots that traverse, but are not limited to, harnessing the combined power of engagement and awareness, genomics, deep phenotyping, and artificial intelligence, whilst equitably scaling their implementation. One digital tool that is currently being implemented in the Global Commission data ecosystem is Cliniface.[10] This 3-dimensionsal facial visualisation and analysis software enables collaboration between clinicians and researchers to advance understanding of facial

characteristics and their relationship with rare diseases and their treatment.

World Economic Forum Precision Medicine Initiative

Precision medicine can be defined as an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.[10] Digital health tools are key enablers of precision medicine. The World Economic Forum (WEF) Precision Medicine Initiative operates in the context of enabling global public–private co-operation and committing to developing principles and frameworks that accelerate the application of science and technology for global public interest, whilst also mitigating for any potential risks of new personalised medicine applications in genomics or big data.[11]

The Initiative focusses on two exemplar implementation domains: rare diseases and cancer. In order to realise the full potential of precision medicine (including related digital health approaches), substantial economic, regulatory, social, and technical challenges to its broad implementation must be overcome. Those identified through the WEF include: 1) generating sufficient evidence; 2) tackling data sharing and infrastructure challenges; 3) reshaping the regulatory environment; 4) adoption of genomic information from research into clinical care; 5) economics of precision medicine; 6) creating payment models involving the gains and risks shared along the value chain; and 7) attaining greater patient and clinician engagement and trust. The WEF supports pilot projects and other initiatives to address, and hopefully relieve, these bottlenecks. One such project is Lyfe Languages.[12] Lyfe Languages is empowering and retaining Indigenous languages, creating more connected communities, and supporting equitable advances in digital healthcare. It is a community engaged and co-designed initiative to deliver Indigenous language translations of the *lingua franca* of precision phenotyping (the Human Phenotype Ontology [HPO]). Through these HPO translations, Lyfe Languages digitises and makes computer-readable descriptions of a condition's manifestations (the phenotype) that are provided directly in an individual's own Indigenous language.

Capturing Phenotype Through the Life and Health System Journey

In medicine, phenotype is a deviation from normal form, function, or behaviour. Phenotype can also be thought of as the voice of the patient and the clinicians describing that patient's condition, statically and dynamically. Tools such as Phenotips,[13] Patient Archive,[14] and Dx29[15] have been developed to facilitate phenotyping, primarily for diagnostic support for rare diseases. Another example is Track.health,[16] which delivers approaches to measure, monitor, and track a patient's journey within the health system, from start to finish.

Primary Care

A patient's medical journey starts and is often housed in primary care. As such, digital health integration within and between primary and specialist care is critical; this is especially true for complex conditions like rare diseases. The UK National Health Service (NHS) Long Term Plan states that every patient will be able to access a digital-first primary care offer by 2023/2024.[17] This, and other primary care initiatives, will need to embrace the challenges and opportunities of caring for people with rare diseases. The norm for people with rare diseases carrying large binders with their medical history and past appointment documentation in paper records can be significantly alleviated by a digital approach.

Other markers to embrace in digital health for rare diseases are: the proposed Innovative Medicines Initiative (IMI) call for newborn screening and digital health tools;[18] conferences that focus on digital health and rare diseases, such as the World Orphan Drug Congress[19] and multiple other digital health initiatives for rare diseases launched by various pharmaceutical companies; and the IMI digital collaboration on rare diseases in Germany.[20]

COMMON THEMES AND NEEDS

Amongst the various rare diseases digital health initiatives there are common themes and requirements. Some of these include: 1) the need for patient engagement and co-design; 2) developing and implementing global technology standards in genotyping, phenotyping (e.g., Phenopackets,[21] a developing open standard for

sharing disease and phenotype information), and diagnostic coding (e.g., ORPHAcodes[22]; "The beginning of wisdom is to call things by their proper name");[23] 3) interoperability, including avoiding 'supersiloes' of patient and clinical/health system data; 4) a focus on equity and tools that can be employed in low-resource environments; 5) real-world data and patient-centred metrics; and 6) global connectivity that can be adapted to suit jurisdictional requirements. Fortunately, the rare diseases community is already heavily invested in many of these elements and is often at the forefront of addressing these issues.

WHY DIGIT-*ALL*?

Rare diseases can cross *all* medical specialties, across *all* the lifespan, *all* aspects of life, and *all* of the globe. Serving the unmet needs of people living with rare diseases requires *all* of us. *All* of the various stakeholders can be digitally connected for more timely, scalable, and sustainable digital health change. It also requires a focus that draws upon and empowers *all* of the community. Time and time again, discoveries in the rare diseases domain are translated to added benefits for people with more common disorders.[24] For instance, one in two new medicines come from rare diseases research; for example, the cholesterol-lowering drugs, statins, are being developed as a result of researching the rare disease, familial hypercholesterolaemia.[24] Specific digital health examples of solutions generated for rare diseases that have then been adapted to serve more common diseases include the pivot of Lyfe Languages from an initial focus on rare diseases to subsequently include the development of resources for novel coronavirus disease-2019 (COVID-19) and immunodeficiency; and the development of the COVID-19 symptom tracker, Covidaware.me,[25] which is based on the rare diseases patient-facing knowledge aggregator rareaware.me.[26] Serving the digital health needs for people living with rare diseases will benefit *all* of us.

CONCLUSION

Rare diseases present a global health challenge with high unmet need. Serving this need will provide opportunities to develop solutions for both rare and common diseases. The context of the challenges and the opportunities continue to evolve, and this is particularly evident in the field of digital health.

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Nutritional Management of Paediatric Crohn's Disease

Nestlé Health Science online symposium, 4th June 2020. This was planned to be presented at the cancelled 6th World Congress of of Pediatric Gastroenterology, Hepatology and Nutrition (WCPGHAN)

Chairperson: Paolo Lionetti[1]

Speakers: Javier Martín-de-Carpi,[2] Rotem Sigall-Boneh,[3] Eytan Wine[4]

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2. Gastroenterology, Hepatology and Nutrition Department, Hospital Sant Joan de Déu, Barcelona, Spain
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Meeting Summary

For many people with Crohn's disease (CD), onset occurs in childhood or adolescence. Treatment for CD has moved from predominantly surgical to, more often, pharmacological. While successful for many, others have tried various medications and combinations without long-term success and, for all, drug treatment needs to be balanced with potential therapy risks. Findings that diet can impact pathogenesis of CD to cause and exacerbate symptoms have inspired studies of dietary interventions. The Crohn's Disease Exclusion Diet (CDED) was developed following the observation that certain dietary components were linked to inflammation and gut dysbiosis found in those with CD. This three-phase diet included two periods of a highly-controlled and prescribed diet, followed by a

maintenance diet in which patients had a wider choice of foods. The diet limited ingestion of foods that may trigger inflammation and/or dysbiosis in CD, such as saturated fats, wheat, carrageenan, and some dairy products, and included healthy choices, such as fruits, vegetables, lean protein sources, and complex carbohydrates. It was nutritionally balanced, science-based, and included foods that were widely accessible. Based on findings from clinical trials and case studies, four experts (Prof Lionetti, Prof Martín-de-Carpi, Mrs Sigall-Boneh, and Prof Wine) discussed the background of CD, current treatment options, the utility of dietary therapies including CDED, and how all healthcare professionals (HCP) looking after children and adolescents with CD should consider the use of diet as part of their therapy.

Introduction to Nutritional Management of Paediatric Crohn's Disease

Professor Paolo Lionetti

CD, a complex condition with a genetic predisposition, can start in childhood or adolescence.[1] Surgery used to be the mainstay for CD but rates have now decreased,[2] possibly because of the use of biopharmaceutical drugs ('biologics'), more tailored use of thiopurines, and earlier disease recognition.[3] However, pharmacotherapy may be limited because each biologic targets only one pathway. As other pathways come into play leading to disease progression, it is not known if, with these agents, the natural history of CD is changed in the long term.[3,4] Dietary therapy with CD may help in this respect.

Diet and the immune response can affect a person's gut microbiota. A diet containing cereals, legumes, and fibre, such as that consumed by children in rural Burkina Faso, promotes a gut microbiota with a wide variety of bacterial species able to extract metabolic energy from ingested plant polysaccharides.[5] This leads to the production of short-chain fatty acids, which help control inflammation[6] and improve epithelial cell energy metabolism in colitis.[7] Comparison of these rural children to those who moved to a more urban environment found the change to a diet higher in calories and lower in fibre altered microbiota composition and lowered short-chain fatty acid levels to become similar to children in urban Italy.[5,8]

However, location (rural versus urban) is less the problem than what is consumed. A 'Mediterranean diet', including fruits, vegetables, whole grains, and seafood, is associated with high microbiome diversity, an intact gut epithelial barrier, and a balanced immune function. In contrast, a 'Western diet', low in fibre and high in total fat, animal protein, polyunsaturated fatty acids, and refined sugars, is associated with alterations in microbiome composition and metabolic activity (dysbiosis), increased epithelial barrier permeability, and loss of immune system tolerance.[9-11]

In the question and answer (Q&A) session, it was asked if everyone should avoid potentially inflammatory foods, such as the additive carrageenan. While there is little evidence regarding recommendations for carrageenan for the general population according to Mrs Sigall-Boneh, Prof Lionetti illustrated how animal and *in vitro* models have shown a significant role for dietary components in gut health. For instance, gut epithelial function and mucous layer composition can be disrupted by carrageenan,[12] emulsifiers,[13] and a high-fat diet.[14]

Prof Lionetti hypothesised that, combined with genetic factors, low-grade inflammation caused by a Western diet can lead to CD development. For example, one large 26-year epidemiological study found CD risk was inversely associated with level of fruit and fibre consumption.[15] As such, controlling diet in those with CD, such as with the CDED, may be useful in managing CD-associated inflammation.[16]

Different Approaches for the Induction and Maintenance of Remission in Paediatric Crohn's Disease

Professor Javier Martín-de-Carpi

Treatment objectives for paediatric CD include achieving and maintaining remission, halting disease progression, and providing adequate nutrition for growth. There is also a focus on limiting potentially damaging medication side effects and reducing surgery and hospitalisations, along with holistic objectives, such as improving quality of life and facilitating support. Of great importance is recognising life-reducing factors associated with CD, including infections, disability, cancer, and bone mineral density problems. While many paediatric HCP do not see such complications, these may occur as the patient grows up with CD.

Following European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Crohn's and Colitis Organisation (ECCO) guidelines, first-line treatment for children with CD is 6–8 weeks of exclusive enteral nutrition (EEN).[17,18] Maintenance therapy may include immunosuppressant drugs, such as thiopurines or methotrexate (MTX); anti-TNFα drugs, including infliximab and adalimumab;[17,19] other biologics, such as vedolizumab or ustekinumab; or combination therapy.[20-24] The benefits of drug therapy must always be considered alongside the risks.

In the long term, some people fail drug therapy at various stages and switching therapies and/or surgery may be required.[25-28] There are also those that unsuccessfully progress down each line of therapy and hit, according to Prof Martín-de-Carpi, a 'non-exit road'.[17] However, he stressed, treatment should not be 'one-directional' and HCP should reconsider previous treatments or re-examine those treatments not used before, including "going back to basics."

CD pharmacotherapy is utilised to stop the immune system reaction and aid the microbiota in blocking inflammation; however, these may not address environmental factors causing CD. Proper dietary interventions could address all of these needs (Figure 1).

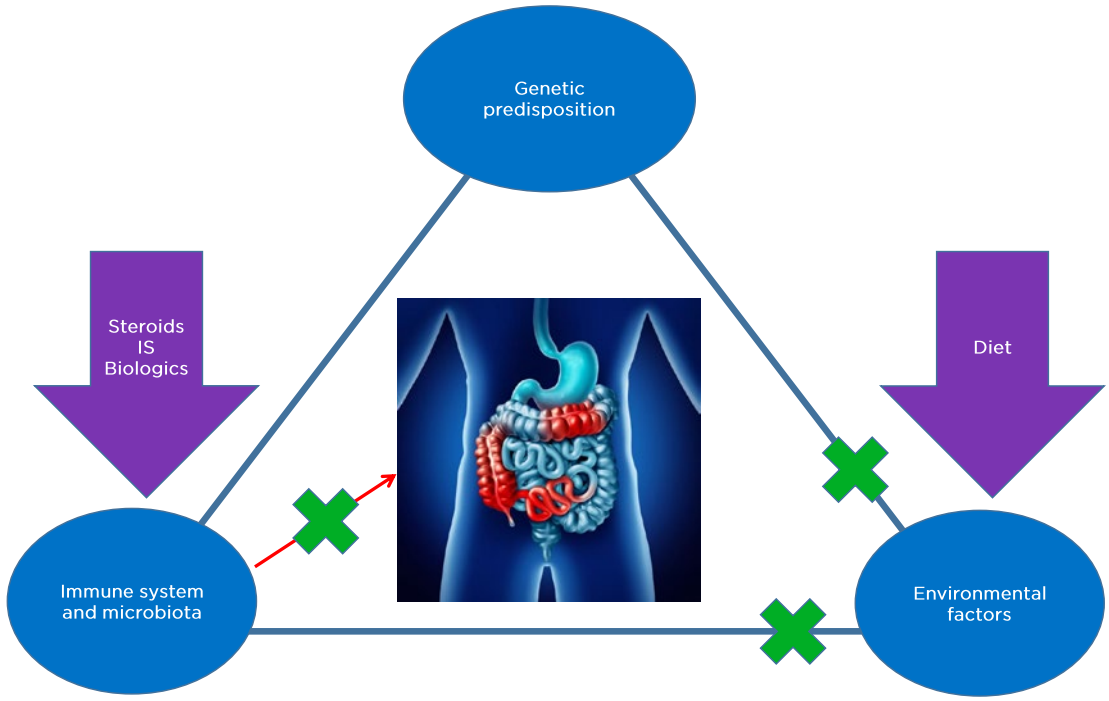

Figure 1: Crohn's disease may have a genetic factor that cannot be changed, but pharmacotherapy and diet can address factors associated with a person's immune system, microbiota, and environment.

IS: Immunosuppressant drugs.

Paediatricians should discuss diet as part of CD treatment because it is a concern for patients and many parents ask about it when their child is diagnosed.[29] Some patients/parents try restrictive, unhealthy diets but, following EEN, previous advice was against dietary modification as no single food was implicated as being entirely involved in CD.[30] The CDED was highlighted by Prof Martín-de-Carpi as being one that people might consider trying because it fits well with the frequent demand by patients and their relatives regarding acceptability and compliance. It is as effective as EEN in achieving clinical and biochemical remission and mucosal healing but superior to EEN in tolerance and compliance.[31-37] Thus, the CDED may constitute a nutritionally balanced long-term strategy for maintaining remission if complied with adequately.

In conclusion, Prof Martín-de-Carpi emphasised how treatment plans need to be accessible and involve tools for patient autonomy to help them manage their daily life with less direct assistance from HCP. Allowing a patient to actively participate in their care could lead to better treatment adherence and lifestyle changes.

Updates in the Dietary Management of Crohn's Disease with the Crohn's Disease Exclusion Diet: Can We Predict a Patient's Response?

Mrs Rotem Sigall-Boneh

As discussed above, CD-associated microbiome changes can lead to inflammation and CD symptoms. For example, dysbiosis is associated with high fat and sugar intake, low fibre intake, and ingestion of gluten, emulsifiers, and taurine. Bacterial pathogenicity, virulence, epithelial translocation, and mucosal adhesion are also associated with many of these dietary factors, as well as with ingestion of maltodextrins, high animal protein intake, and low intake of resistant starch.[31]

CDED, developed in 2011 by Prof Arie Levine, is a proven dietary plan with a high level of evidence for efficacy.[16] CDED excludes potentially proinflammatory dietary factors to help reduce inflammation and improve the microbiome balance. It comprises three phases defined by which foods, and how much, can be consumed in each. Phases 1 and 2 are 6 weeks each and are designed to induce remission, with Phase 3 set as a continuum with more flexibility in the diet, adapted for maintenance of remission. Progressive exposure, where at each phase more foods are allowed, makes it easier for long-term compliance. CDED is balanced in nutritional needs, is palatable, and includes allowed foods (some of which are highly recommended), excluded foods, and those that may require exclusion or reduced exposure depending on the individual.[31] The diet uses foods that can be widely accessed and includes recipes and a support programme (ModuLife, Nestlé Health Science, Switzerland).

CDED foods include fruit, vegetables, resistant starch, high-quality lean protein sources, complex carbohydrates, and healthy oils. Food choices define consumption of low or no amounts of inflammatory-linked components, including animal and saturated fats, taurine, wheat, haem/iron, emulsifiers, maltodextrins, carrageenan, sulphites, and dairy products.[31] In Phase 1, CDED foods constitute approximately 50% of a patient's energy requirements, with 50% from partial enteral nutrition (PEN). In the Q&A, Prof Lionetti discussed how tube-fed EEN was originally essential because the formula was "unpalatable," but more recent polymeric formulas may be taken orally because "the taste is much better and accepted by both adults and children." A similar formula is used as part of the CDED to complement nutrition and energy needs. Moreover, during the Q&A, Mrs Sigall-Boneh discussed how in Phase 1, fibre is limited as a result of inflammation that might cause narrowing of the intestine and might lead to abdominal pain; however, at later stages exposure to fibre is recommended if no stricture is present. In Phase 1, refined rice is allowed if cooked with a lot of water to reduce potential arsenic exposure. In Phases 1 and 2, frozen and processed foods are discouraged because some have additives; however, some can be introduced in Phase 3 if tolerated.

CDED studies have found CDED to have better tolerance and adherence than EEN[16,32] and have success in complicated situations, for example in patients who do not respond to biological therapy.[33]

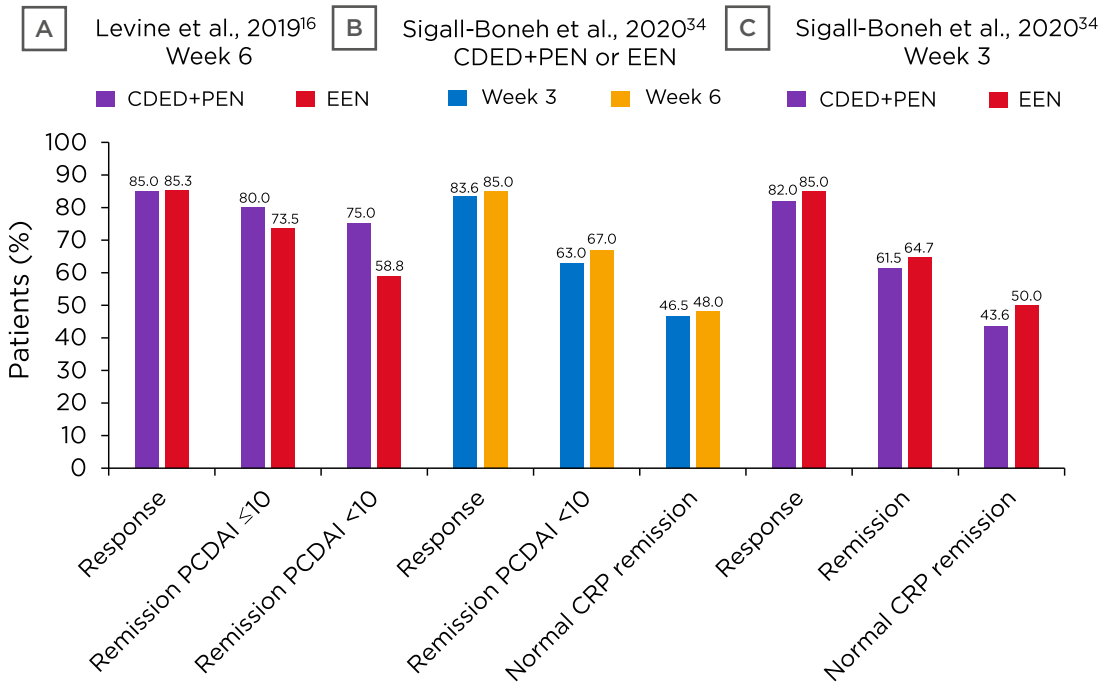

Figure 2: A) CDED+PEN at Week 6 response and remission; **B)** response, remission, and CRP levels following dietary therapy (CDED+PEN or EEN); **C)** CDED+PEN or EEN at Week 3 response, remission, and CRP levels.

CRP: C-reactive protein; CDED: Crohn's Disease Exclusion Diet; EEN: exclusive enteral nutrition; PCDAI: Paediatric Crohn's Disease Activity Index; PEN: partial enteral nutrition.

Adapted from Levine et al.;[16] Sigall-Boneh et al.[34]

In 2019, Levine et al.[16] published the first randomised controlled trial that investigated CDED+PEN (n=40) compared to EEN (n=38) in children aged 4–18 years with mild-to-moderate luminal CD, defined by a Paediatric Crohn's Disease Activity Index (PCDAI) score between 10 and 40. By Week 6 (Figure 2A), there was a high and comparative response rate with both diets and many participants achieved a PCDAI score ≤10, with some having a PCDAI score <10.

As dietary treatment can lead to remission, Mrs Sigall-Boneh posited that early response might be used to predict this. An assessment of Levine et al.'s[16] data found that most participants responded to dietary therapy by Week 3, with many having a PCDAI score <10 (Figure 2B). Similar response/remission rates were shown between diets at both Week 3 and Week 6 (Figure 2C), along with a significant reduction in the inflammatory biomarker C-reactive protein (CRP) in both groups (Figure 2B and Figure 2C).[34]

In this analysis, 75.4% of those who achieved response at Week 3 showed remission at Week 6. Of those with a PCDAI score <10 at Week 6, 94.0% achieved response and 81.6% achieved remission at Week 3. As such, remission at Week 3 was found to be predictive of remission at Week 6 (odds ratio [OR]: 6.37; 95% confidence interval [CI]: 1.600–25.000; p=0.008) and Week 12 (OR: 4.5; 95% CI: 1.235–16.476; p=0.023). Week 6 remission was negatively predicted by poor compliance (OR: 0.75; 95% CI: 0.012–0.460; p=0.006).[34]

It may be that Week 3 response results can be used as a diagnostic tool such that for responders, assurance can be given that they will very likely continue to respond to CDED, potentially for the rest of their lives. If a person does not respond at Week 3, with no other biological markers indicating signs of improvement, it may indicate the need to abandon the diet and initiate other treatments instead of persisting to Week 6.

In the Q&A, it was suggested that response factors could include the inflammatory protein faecal calprotectin (FCP) and microbiome analysis. However, as discussed by Prof Wine, Levine et al.[16] found FCP response to be slow and, while microbiome changed to a degree with dietary therapy, microbial balance predominantly reappeared when food was reintroduced to EEN, but not with Phase 2 CDED.

The Q&A also raised the question of whether there were comparison studies regarding mucosal healing with CDED or drug therapy. The panel discussed how studies that have examined this, including a comparison of CDED with steroids, were ongoing. Prof Wine emphasised that it was important to consider not only how quickly a treatment works, as may be found with steroids, but how safe it is long term.

In conclusion, Mrs Sigall-Boneh emphasised how therapeutic strategies should be personalised and include the option of dietary modulation. She suggested that short-term use of dietary therapy may be warranted in some to identify dietary response and that dietary therapy can be used as a standalone therapy, a bridge to biological therapy, or as an adjunct to medication.

From Theory to Practice: Clinical Use of Crohn's Disease Exclusion Diet in Real-Life Cases

Professor Eytan Wine

The aforementioned clinical trial excluded children using steroids or biologics and those with perianal or primary colonic disease.[16,35] Prof Wine posited that other CD cohorts could benefit from CDED, such as those with severe luminal CD, individuals who are refractory to drug therapy, or for those where CDED might be beneficial beyond 12 weeks. Recent studies have highlighted the important role of dietary therapies for those with CD in settings different to clinical trials[36,37] and ongoing studies are focussing on more severe patients.

Case Presentations

Case 1

Case 1 highlighted the utility of CDED+PEN for severe CD. A 12-year-old male with a genetic predisposition experienced escalating pain over 3 months, bloody diarrhoea, weight loss, mouth sores, and fatigue. His PCDAI was 45 and he had high inflammatory markers including CRP (24.5 mg/L) and FCP (3,378 µg/g) at diagnosis. Endoscopy revealed panenteric disease and biopsies were positive for granulomas (indicating immune system activation and inflammation). CD was severe in the terminal ileum where ultrasound revealed a long, thickened segment (3.5–4.0 mm) with fat proliferation and stratification loss.

Two weeks of EEN achieved almost complete remission with no pain, little diarrhoea, normal CRP (<0.5 mg/L), and a PCDAI of 10. While usual practice would be to continue EEN, the patient undertook Phase 1 CDED+PEN and was happy to resume eating at least some food. Complete clinical remission was achieved at 6 weeks, with a PCDAI of 0, normal CRP (0.2 mg/L), and normal terminal ileum thickness (0.9–1.4 mm). After 6 weeks of Phase 2 CDED, PCDAI was 0, CRP was <0.2 mg/L, and FCP was 229.4 µg/g. The patient continued onto Phase 3 maintenance CDED and remained in remission 22 weeks after initial treatment.

Reflecting this case, one Q&A query posited whether CDED could be used as maintenance therapy alone. Data are currently only based on experience, though trials are ongoing. Studies of maintenance CDED are "challenging," explained Mrs Sigall-Boneh, "as patients can have some 'free' meals and choose what to eat. We educate the patient to maintain some kind of restrictive diet but still go out and enjoy a normal life and find the balance." Prof Wine added that: "There is some fatigue for patients on a maintenance diet even though it's more liberal. There are limitations and certainly for some children it's more difficult while some love it."

During the Q&A, Prof Wine shared: "Even if we're getting a partial response [with CDED] and you get along with less adjuvant therapy, that's an accomplishment. If you can get the effect you get with EEN of not needing steroids and allowing us to complete the workup and start the patient

on another therapy in a better situation, that's a good enough reason to do it." The following cases highlight this.

Case 2

Case 2 examined CDED+PEN in someone who had lost response to biologic treatment. This 22-year-old male was diagnosed with CD aged 16 years old and was treated with infliximab plus MTX, then switched to adalimumab plus MTX when infliximab response was lost. He achieved clinical remission including partial mucosal healing; however, there was noticeable loss of response to adalimumab when MTX was discontinued.

On presentation, the patient reported frequent diarrhoea, weight loss, anorexia, and resumption of symptoms over 6 weeks. Investigations revealed elevated CRP (19 mg/L) and FCP (604 µg/g). With adalimumab retained, the patient was given Phase 1 CDED+PEN. By 6 weeks, his CRP level normalised, he had no pain or diarrhoea, and was nearing remission so he proceeded to Phase 2. Week 12 assessment showed reduced FCP (228 µg/g) and normal CRP. Unfortunately, as a student away from home, while he continued taking adalimumab, he could not maintain the diet and he relapsed. Importantly, this case showed that dietary intervention can be successful in addition to biologics, as supported by similar case reports,[3] and highlighted the importance of compliance in achieving effectiveness of dietary management of CD.

Case 3

Case 3 discussed refractory disease with an indication for surgical intervention. A 15-year-old female was diagnosed with severe panenteric CD aged 5 years old. She was initially treated with infliximab plus azathioprine (later switched to MTX), then switched to adalimumab plus MTX following loss of response. Unfortunately, her symptoms, including abdominal pain, vomiting, and diarrhoea, were ongoing and she was hospitalised with a severe relapse. At this point, she showed severe panenteric stricturing, inflammation (an erythrocyte sedimentation rate of 90 mm/hour), and adalimumab trough levels of 15 µg, suggesting pharmacodynamic failure.

Remission was achieved after 2 weeks EEN and she commenced 12 weeks CDED+PEN (Phases 1 and 2). While this therapy brought remission, she felt it was too difficult to continue with. Ustekinumab therapy was initiated but she was nonresponsive after 6 months and was hospitalised. Examination revealed numerous deep ulcers from rectum to caecum: a 15 cm thickened bowel loop with strictures and proximal loop inflammation (total 30 cm). She had an erythrocyte sedimentation rate of 76 mm/hour and indications of microcytic anaemia.

This patient had a clear indication for surgery, including resection plus ileostomy, but she consented to retrying dietary therapy with EEN, followed by 12 weeks of CDED+PEN, to try to avoid surgery. Colonoscopy following therapy showed no inflammation with a normal colon, though because the ileum could not be intubated, a small segment was removed. Following surgery, adalimumab was restarted, and the patient remained in remission after 2 years.

During the Q&A, adalimumab re-administration was queried because the patient had previously lost response to it. Prof Wine explained that: "This treatment was the most effective over time. The rationale was that the diet and removal of the strictured segment would lead to a debulking effect, get inflammation under control, and get the patient into remission to set them up to have more success with the treatment they were on before." Further, it was asked what could be done if a symptom flare-up occurred during Phase 3 maintenance CDED. Case 3 highlighted how, even without maintenance therapy, a person can return to Phase 1 CDED and it can be used alongside drug therapies to help induce and retain remission.

Prof Wine concluded by suggesting that there are many people with CD for whom CDED can be used at any disease stage or location, as shown in Figure 3.

To highlight one example of a potential CDED candidate, the Q&A considered people with strictures. Prof Wine discussed how CDED Phase 1 is low in fibre because of concern from stricturing disease and those with stricturing disease were excluded from the RCT[16] as likelihood of success was lower.

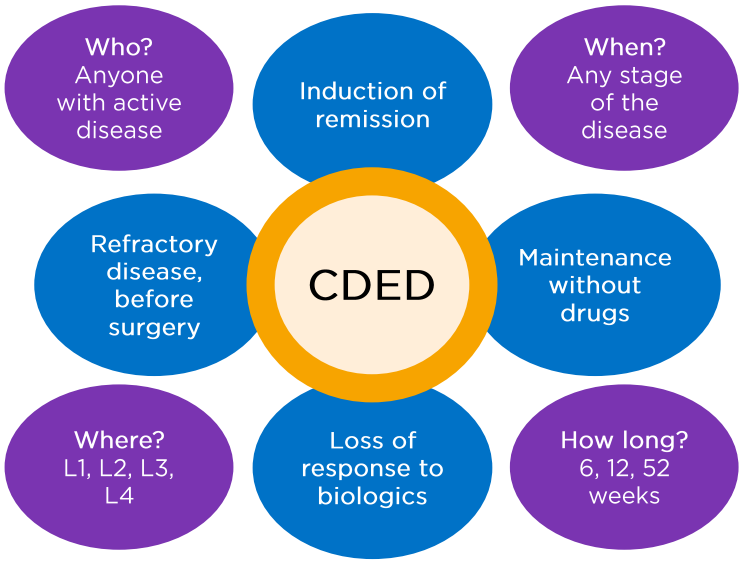

Figure 3: Who is Crohn's disease exclusion diet therapy for?

CDED: Crohn's Disease Exclusion Diet.

Adapted from Prof Arie Levine, personal communication.

Prof Martín-de-Carpi agreed that these patients "may not be the best candidate for CDED; however," he continued, "if there is a long delay in diagnosis and clear MRI images of strictures and previous dilations, it's reasonable to try CDED or EEN… [with] close follow-up to try and get a full response." Although, he advised that: "If you don't hit the target you're looking for, change to a treatment that's more effective."

Prof Lionetti discussed how there can be problems discriminating between inflammation and fibrosis. However, he posited that a liquid diet could help prior to surgery to limit strictures. This position was endorsed by Mrs Sigall-Boneh, whose patients often have EEN for 1–2 weeks prior to surgery. Prof Wine shared that: "There are some [with strictures] I'd try with a liquid diet in the first week or so and, if we see improvement, we would use CDED to try and settle inflammation." This has the advantage of avoiding medication-related side effects. Prof Martín-de-Carpi agreed that: "We all have some patients you think are going to require surgery, but you can control the disease and diminish inflammation so it's worth trying." Prof Wine reported that there are, however, "some cases where we don't hesitate to go to surgery or use biologics."

Conclusion

While pharmacotherapy for CD has reduced the need for surgery, there are concerns for the utility of single-target medications and adverse event profiles. Dietary therapy, such as with CDED, is efficacious for many and could be trialled for a wide range of patients.

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Interview

Dr Krishnan Ganapathy

Member of the Board of Directors of Apollo Telemedicine Networking Foundation (ATNF) and Apollo Telehealth Services; Past President Telemedicine Society of India (TSI) and Neurological Society of India (NSI); Emeritus Professor at The Tamil Nadu Dr. M.G.R. Medical University, Chennai, India

Q1 At present, you are the President of the Apollo Telemedicine Networking Foundation (ATNF). What is the aim of ATNF, and how did you come to be involved with the Foundation?

It all started on the night of 16th September 1996, I had just finished delivering an institute lecture in the Indian Institute of Technology Kanpur (IITK), a globally recognised institution. Just after the question and answer session ended at 9 pm, Dr K. Srivathsan from the Department of Electrical Engineering at IITK, insisted I have dinner with him. He introduced me to the word 'telemedicine'. Together, we prepared a project report from 11 pm to 4 am. I then commenced a love affair which over the last 24 years, has taken its toll. My legally wedded wife is often relegated to number three. Initially, I was wedded to neuro surgery and now it is telehealth. Having started the first stereotactic radiosurgery unit in South Asia, and as Secretary of the then 2,200-strong Neurological Society of India (NSI), conventional wisdom dictated that I should continue to focus my skills and energy completely on what I was trained for, namely neurosurgery. However, deep down was a nagging feeling: "Was there not something else which I could do to help more than a few thousand neurosurgical patients?" And then the story began. I took the road less travelled by and the rest, as they say, is history! In fact, I embarked on making geography, history, and distance meaningless!

Dr Prathap Reddy, Founder and Chairman of the Apollo Hospital Group, Chennai, India, visionary Nostradamus that he is, gave me a lot of support. On 24th March 2000, President Bill Clinton formally commissioned the world's first Very Small Aperture Terminal-enabled village hospital in Aragonda in Andhra Pradesh (birthplace of Dr Reddy). In 2001, the Apollo Telemedicine Networking Foundation (ATNF) was formally established as a not-for-profit Section 25 company. Taking modern healthcare to remote areas using technology was the mission of ATNF. Between my neurosurgical commitments, I spent time and effort to help a skeleton staff of four full-time employees to achieve what in 2001 appeared preposterous: remote consultation! Every opportunity to put, then hardly existent, Indian telemedicine on the world map was utilised.

Q2 Which patients are set to benefit the most from the integration of telemedicine into health systems?

I honestly do not believe that there is one set of patients who will benefit more from the integration of telemedicine into the health system. In fact, there is not a single individual who would not benefit from remote healthcare. The very words 'patient' and 'telemedicine' may

be inappropriate! I would prefer use of the words 'telehealth' and 'beneficiary'. We should start promoting wellness, the eWay! Keeping everyone healthy remotely using technology is my mantra for the future. We can start by promoting health literacy. Providing dependable knowledge on a smartphone will lead to people's empowerment and a reduction of preventable diseases. Under Ayushman Bharath, the world's largest Universal Health Coverage for 500 million Indians, 150,000 telehealth enabled wellness centres will be functional throughout India. Theoretically, every single individual, healthy or sick, rich or poor, urban or rural, educated or not, can benefit in different ways through 'contactless' medicine. Physical distancing is here to stay. This should not be mistaken for social distancing or clinical distancing. Distancing is a term that should not exist for those deploying virtual healthcare, we are always there 24/7 on a small or large screen!

Q3

What are the challenges facing telemedicine today?

Perhaps in one way, the challenges facing telemedicine today are far less and can be addressed. When I first embarked on telemedicine 24 years ago, the very word was unfamiliar to most. It took a decade of intensive persistent evangelisation to create the semblance of an awareness. The second decade was spent in achieving a behavioural modification and technology acceptance among all stakeholders in the ecosystem. During the last four years, thanks primarily to public–private partnerships, revenue-generating business models have started to become available. The single most important challenge facing telemedicine is addressing the question, 'WIIIFM: what is in it for me?' WIIIFM is different for each stakeholder. The COVID-19 pandemic has made the entire globe realise that today, distance is meaningless. Physical distancing will be the norm. The world has turned upside down. I foresee that the challenge will no longer be to convince the healthcare provider and the beneficiary that telemedicine has advantages over face-to-face in person visits. Today, the challenge is to very quickly customise and make available a cost-effective, need-based, user-friendly, technologically efficient, and secure telehealth system which is compliant and adherent to newly formed regulations. The telemedicine system must be future ready and culturally sensitive. Insurance companies in India have already started recognising telemedicine for reimbursement. Revenue generation is critical for ensuring self-sustenance. Necessity is the mother of invention. Telehealth now has to take centre stage. It can no longer be lurking in the periphery. There is an opportunity in every crisis. The challenges facing us in introducing telemedicine are not insurmountable as there is a pressing need not tomorrow, not today, but yesterday. This universal demand alone makes all challenges pale into oblivion.

"There is no doubt whatsoever that in the last 4 months, developments in the face of COVID-19 have achieved what we could not for 24 years. "

Q4

Teleradiology is thought of as one of the fastest moving fields in telemedicine. In your opinion, why do you think there has been a lot of progress in this area?

Imaging procedures are growing 15% annually against an increase of 2% in the radiologist population. Worldwide, there is a shortage of radiologists, particularly in subspecialties. The phenomenal additions to technology and cost reduction make it possible to have CT scans even in towns in India. Simultaneously connectivity in suburban and rural India is getting better. Radiologists, therefore, are extending their reach, some even to other continents! Telerad Tech (Bengaluru, India) provides 24/7 teleradiology services to 1,500 installations in 31 countries. This is particularly important when a subspecialist such as an MRI radiologist, neuroradiologist, paediatric radiologist, or musculoskeletal radiologist is needed. These subspecialists are generally only available in large metropolitan cities during daytime hours. Teleradiology allows for trained specialists to be available 24/7 in different time zones. We embarked on teleradiology only a few years ago. We set up 110 teleradiology centres in suburban and rural Uttar Pradesh, the largest state in India (population of 200 million), in the most challenging areas under a public–private partnership. Today, tens of thousands get imaging studies done without having to travel long distances.

Q5 You are reputed as having pioneered the introduction of telemedicine into India. What advice do you have for someone wanting to establish a telemedicine programme?

Be future ready. Learn from the mistakes of your predecessors. Remember that no telemedicine programme can be sustained unless it is revenue-generating. Technology is only a means to achieve an end not an end by itself. With more access to technology, do not forget that you are a doctor first and last. Tender, loving care can be bestowed virtually. Empathise and sympathise with your patient on the screen (small or big). Wipe their tears. Listen to them. Find out what they want. Answer all their queries. Technology is the least important. Context is critical. Stop a teleconsultation if your gut feeling suggests a face-to-face interaction. Adhere and comply with all changing regulations. We are in a stage of transition. All transitions offer great opportunities. Telemedicine, like medicine itself, is not black and white. It is still various shades of grey. Look back to 10th March 1876, when Alexander Graham Bell made the world's first telephone call, a request for medical help, "Watson come here, I want you," after having spilt battery acid on himself. We now have a diamond spoon in our mouths. COVID-19 is providing us with tens of thousands of virtual non-COVID-19 patients! You cannot ask for a more opportune time to start telemedicine. A strand of RNA 120 nm is your most efficient Chief Transformation Officer.

Q6 What impact has the COVID-19 pandemic had on the trajectory of the integration of telemedicine in day-to-day clinical practice?

Following notification of COVID-19 as a pandemic on 25th March 2020, the Ministry of Health for the Government of India notified Telemedicine Practice Guidelines for the country. This had been pending with the government for a long time. Because of uncertainty over whether practising telemedicine was legal in India, many doctors were not deploying it. Following this, several thousand doctors have attended orientation programmes conducted by the Telemedicine Society of India (TSI) and other organisations. I have personally given 29 webinars in the last 4 months on the role of telehealth in the present situation. Over 34,000 doctors have attended these sessions. One webinar was attended by 11,775 doctors. Another webinar (http://kganapathy.com/covid.html) was for eight countries and 808 attended. The interest has been unbelievable. Simultaneously, hundreds of hospitals in the public and private sectors are offering free teleconsultations for screening of COVID-19 patients. Health insurance companies have recognised telemedicine services for reimbursement. Contactless medicine is now being accepted. It appears that the 'work from home' culture will include doctors as well! The world has turned upside down. Delivery of healthcare services is being re-examined. In every crisis there is an opportunity. Telehealth is here to stay and will take centre stage after having been in the periphery for the last 24 years. There is no doubt whatsoever that in the last 4 months, developments in the face of COVID-19 have achieved what we could not for 24 years.

Q7 There is debate over the robustness of cybersecurity measures for patient data in telemedicine. What is your opinion on this?

We concede that privacy and security of patient data are very important. Yes, 200 million healthcare records have been reported to have been compromised in the USA alone. The later entry of India in using electronic medical records (EMR) has ensured that we don't piggyback, we don't even leapfrog (after all, how far can a frog leap!): we pole vault. Ethical, Legal and Social Issues (ELSI) guidelines regarding data ownership of EMR have been notified. Protected health information, data ownership, data access and confidentiality, EMR preservation, and patient-identifying information have all been addressed in great detail under existing Indian laws, including the Information Technology Act 2000 and its amendments. The stakeholders understand the importance of cybersecurity; however, sometimes I feel that we are getting paranoid about cybersecurity in healthcare. Yes, it is important but if we wait for a totally fool-proof secure system EMR and hospital information systems would never see the light of the day and the cost would be astronomical. Even the White House can be hacked! It depends how badly the hacker wants the information. I have always felt that there is a cultural difference between India and the West so far as hacking health records is concerned. We are not primarily a society that will litigate at the drop of a pin. Trust still is a part of our vocabulary.

Tumour-Associated Hypoxia: Can We Give Chimeric Antigen Receptor T Cells More Breathing Space?

EDITOR'S PICK

CAR-T cells are already a targeted immunotherapy. The development of CAR-engineered T cells that are exclusively expressed or activated under conditions of profound hypoxia, a feature of solid tumours, would increase the efficiency of this approach. In dual-targeted CAR-T, the therapy would utilise the antigens from one side and the hypoxia from the other side.

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Abstract

Immunotherapy using chimeric antigen receptor (CAR)-engineered T cells has encountered important limitations in the transition of their use from liquid to solid tumours. Success is dependent upon T-cell trafficking to, and functional persistence within, tumours that often present a metabolically

and immunologically hostile microenvironment. Moreover, CAR targets that are tumour specific are extremely scarce. To address these issues, several strategies have been proposed to improve both tumour selectivity and safety. One approach involves the engineering of CAR-T cells that only deploy their effector function at tumour sites. Conceptually, a solution for this exploits the oxygen-limited nature of advanced tumour deposits through the engineering of CAR that are exclusively expressed or activated under conditions of profound hypoxia. T cells have a complex inter-relationship with oxygen, which also needs to be factored into the refinement of these technologies. Ideally, oxygen-sensing CAR should only function when oxygen tension is below 2%, as is commonly the case in solid tumours but rare in healthy tissue. Successful advancement of such technologies presents opportunities for solid tumour immunotherapy because it should broaden the target repertoire that may safely be exploited in this context.

INTRODUCTION: THE CHALLENGE OF SOLID TUMOURS

Chimeric antigen receptors (CAR) are fusion molecules that couple the binding of a native tumour-associated target to the delivery of a bespoke T-cell activating signal. To extend the success of CAR-T cells from the haematological to the solid tumour setting, a number of key challenges have hindered progress.[1] Firstly, there are numerous physical constraints that hinder CAR-T cell recruitment, activation, proliferation, and persistence within solid tumour deposits. Secondly, CAR-T cells that do manage to navigate into the solid tumour microenvironment must then operate in the face of the adverse metabolic conditions at the site, notably hypoxia, low pH, elevated lactate concentration, and deprivation of nutrients such as glucose and amino acids.[2] Even in the setting of more amenable haematological malignancies such as B-cell leukaemias and lymphomas, important safety concerns include potentially lethal cytokine release syndrome and neurotoxicity. In solid tumours, the relative absence of truly tumour-selective targets means that on-target, off-tumour toxicity is a further major concern that has caused morbidity and death of some patients.[3]

Numerous strategies have been developed to improve the therapeutic index of CAR-T cell immunotherapy.[4,5] These include the intrinsic modification of CAR architecture, engineering of systems to control CAR-T cell persistence, and use of loco-regional delivery systems to minimise systemic exposure to these cells.[6,7] Exemplifying this, a recent clinical trial has demonstrated the safety and efficacy of mRNA-transfected c-Met-CAR-T cells following their intratumoural administration, thereby achieving transient expression of a potentially toxic CAR primarily at the site of disease.[8] Similarly, intratumoural delivery of panErbB CAR-T cells has been safely employed in the treatment of patients with relapsed or refractory and locally advanced head and neck cancers.[9] More complex strategies that set out to ensure safety include the use of suicide genes,[10] inhibitory CAR,[11] dual antigen-targeted systems,[12] combinatorial SynNotch CAR,[13] and switchable CAR.[14-17] To maximise success and safety requires a system that tightly restricts the activation of CAR-T cells to the solid tumour microenvironment.

In 2019, the Nobel Prize in Physiology or Medicine was awarded jointly to William Kaelin, Peter Ratcliffe, and Gregg Semenza for their work which characterised how cells sense and adapt to ambient oxygen levels. Given the profoundly hypoxic nature of many solid tumours, this paper considers the potential effects of hypoxia on CAR-T cells and how these may be harnessed to enhance the tumour specificity of these cells.

HYPOXIA OF THE TUMOUR MICROENVIRONMENT

Hypoxia refers to a low concentration of oxygen and is a frequent attribute of solid tumours.[18] It commonly results from rapid tumour growth that outstrips an inadequate and often abnormal blood supply. Hypoxia has been associated with tumour cell invasiveness, metastasis, epithelial to mesenchymal transition, maintenance of cancer stem cells, and resistance to both radiation and cytotoxic chemotherapy.[19-21] While oxygen tension in normal tissues is generally ≥5% and reaches approximately 14% in the lung, levels fall to below 2% in solid tumours and in

chronically inflamed tissues.[22] Oxygen plays a fundamental role in many metabolic processes and cells must adapt nimbly to fluctuating levels of this nutrient in order to remain viable.[23-25] The key regulators of oxygen homeostasis are hypoxia-inducible factors (HIF): HIF-1, HIF-2, and HIF-3.[26] While the importance of HIF-3 is less apparent, HIF-1 and HIF-2 operate as transcriptional regulators of an overlapping set of genes that mediate the hypoxic adaptive response.[27]

HIF are heterodimers comprising a stably expressed β subunit and a hypoxia-dependent α subunit. At normal oxygen levels, HIF-1α is rapidly degraded. This process is dependent upon the hydroxylation of proline residues found in the oxygen-dependent degradation domain (ODD) of HIF-1α by the enzyme, prolyl hydroxylase. Upon hydroxylation, the von Hippel–Lindau protein (VHL) binds and orchestrates HIF-1α ubiquitination followed by proteasomal degradation.[26] Conversely, under hypoxic conditions, the HIF-1α protein accumulates, translocates to the nucleus, and dimerises with the HIF-1β subunit, forming the HIF-1 complex. The latter can then bind to hypoxia-responsive elements in the promoter region and increase transcription of specific hypoxia-induced genes.[28]

It has been argued that hypoxia represents the best validated cancer-associated feature that has yet to be exploited clinically.[29] A wide number of experimental treatments are focussed on harnessing tumour-associated hypoxia, including prodrugs[30] and HIF inhibitors.[31] CAR-T cell immunotherapy strategists also need to consider the challenge and potential opportunity presented by the profoundly hypoxic nature of many solid tumours. Recent studies have investigated the effects of oxygen deprivation on CAR-T cells and some research groups have gone further to exploit hypoxia by developing oxygen-sensing CAR-T cells.

CAR-T CELLS AND HYPOXIA

Little is known about how CAR-T cells behave in an hypoxic environment. However, hypoxia is known to exert several effects on unmodified T cells, including the induction of apoptosis, compromised activation, and upregulated expression of inhibitory receptors.[18,32] Nonetheless, there is evidence that these inhibitory effects of hypoxia are not generalisable for all T-cell subsets. It has been argued that hypoxia favours the differentiation of Th9, Th17, and Th22 cells while hindering the differentiation of Th1 and cluster of differentiation-8 (CD8) effector T cells, while data on the effect on regulatory T cells are conflicting.[18,33] Moreover, Xu et al.[34] showed that hypoxia enhanced the proliferation, survival, and cytolytic activity of effector memory T cells, in contrast to its inhibitory effects on naïve and central memory T cells. This effect was attributed to elevated glycolytic activity, accompanied by enhanced HIF-1α expression. Hypoxia enhanced the cytotoxic activity of a CAR directed against the GD2 ganglioside, associated with increased degranulation of the CAR-T cells upon target recognition.[34] Furthermore, antigen-specific CAR-T cells that were mobilised into hypoxic tumour areas were more proliferative than CAR-T cells in normoxic areas, when evaluated in two *in vivo* models.[34,35]

Berahovich et al.[36] also explored the effects of hypoxia on CAR-T cells targeted against B-cell maturation antigen (BCMA) and CD19. They observed that hypoxia diminished the expansion of both CAR- and non-transduced T cells.[36] Differentiation of both BCMA and CD19 CAR-T cells was also affected, switching from central memory towards an effector memory phenotype. They also observed an increase in the CD4:CD8 CAR-T cell ratio, suggesting that CD8+ CAR-T cell expansion is more dependent on oxygen.[36] While cytolytic activity of CAR-T cells that were expanded in 5% oxygen was increased, these cells manifested a significant reduction in the production of interferon-γ, IL-2, IL-6, and granzyme B, with programmed cell death protein 1 levels unaffected compared to CAR-T cells expanded in atmospheric oxygen levels.[36] Consistency of findings between CD19 and BCMA-targeted receptors suggests that the effects of hypoxia were CAR-independent.

These *in vitro* data provide an insight into the way CAR-T cells function in a microenvironment restricted of oxygen, simulating that found in the tumour microenvironment. These findings also provide the basis for design of advanced CAR systems that actively exploit tumour-associated hypoxia for therapeutic gain.

RESTRICTING CAR-T CELL FUNCTION TO HYPOXIC ENVIRONMENTS

The first attempt to exploit the hypoxic tumour microenvironment to regulate CAR expression was reported by Ang et al.[37] In an abstract publication, they described the use of the 'Sleeping Beauty' transposon system to express a second generation (CD28+CD3ζ) anti-CD19 CAR fused to an ODD to restrict CAR expression to conditions of hypoxia. No expression of the ODD-containing CAR construct was seen at atmospheric oxygen (referred to as normoxia hereafter), while cell-surface CAR expression was detectable following the transfer of cells to 1% oxygen.[37]

The following year, this group described an oxygen-sensitive c-Met-specific CAR that utilised a fused ODD domain derived from HIF-1α (Figure 1A–B).[38] Like its predecessor, this CAR was expressed using the Sleeping Beauty transposon system. Cell-surface expression of the CAR and cytolytic activity against c-Met-engineered target cells were both strictly dependent upon hypoxic conditions.[39] In a related approach, the clinical-stage biopharmaceutical company, Cellectis, Paris, France, described a method to create a multi-input signal-sensitive T cell. The method involved the application of a logic AND gate whereby two independent stimuli as input signals are required for CAR activation to occur. To exploit hypoxia as one such input signal, the CAR was engineered to contain an ODD. By this means, cognate ligand engagement was restricted to conditions of hypoxia.[40]

The hypoxia-exploiting CAR described in this paper had a multichain (mc)CAR architecture (Figure 2). The latter was based on the high-affinity IgE receptor (FcεRI),[41] a configuration that had earlier been designed to regulate CAR activity in a small molecule-dependent manner.[14] The mcCAR was engineered by replacing the extracellular domain of the α chain for a single-chain variable fragment fused to a hinge domain from the CD8α chain. The native domain of the β chain was substituted with a 4-1BB domain while the CD3ζ chain was appended to the γ chain endodomain. Three CAR were designed as follows. HIF-CAR1 was engineered by fusing the ODD sequence (amino acids 380–603) of HIF-1α to the α chain of the mcCAR, thereby conferring oxygen-dependent stability on the fusion receptor. In HIF-CAR2, the N-terminal domain containing the key proline residue P402 was added (amino acids 344–417), while in HIF-CAR3, the C-terminal domain containing the key proline residue P564 (amino acids 530–652) was added. As indicated above, these residues have previously been shown to interact with the VHL tumour-suppressor protein and to be hydroxylated in conditions of physiological normoxia, ultimately leading to protein degradation.[42-44]

To demonstrate that oxygen could be used to turn on the switch, T cells were electroporated with mRNA encoding for the CAR-ODD for comparison with a control CAR that lacked an ODD. After incubation for 20 hours under hypoxic conditions, cell-surface expression of HIF-CAR1 and HIF-CAR2 were enhanced, while the HIF-CAR3 (for unclear reasons) and the control CAR responded minimally. To demonstrate that the switch was reversible, T cells were transferred from hypoxia to normoxia for 6 hours. Cell-surface expression of HIF-CAR1 and HIF-CAR2 were both successfully negated, whereas no change in expression was observed for the control CAR.

To further evaluate the dynamics of the system and the rate of CAR decay upon removal from hypoxia, T cells were electroporated and placed in normoxia for 6 hours to recover. Next, hypoxia was applied for 16 hours and cell-surface CAR expression was monitored over 6 hours of reoxygenation thereafter. Cell-surface CAR expression decreased by 80% with a lapse of 2 hours for both HIF-CAR1 and HIF-CAR2 and this remained stable for the next 4 hours. In concordance with previous experiments, control CAR and HIF-CAR3 did not respond upon reoxygenation.

To assess cytolytic activity, control or HIF-CAR1 T cells were co-cultured at different effector-to-target ratios for 16 hours with Daudi cells (a human Burkitt lymphoma B-cell line), either in normoxic or hypoxic conditions. Tumour cell killing was observed in both hypoxic and normoxic conditions at effector-to-target ratios of 5:1 and 10:1, respectively. While enhanced killing was seen in hypoxic conditions, leakiness of the system was suggested by the detection of cytotoxic activity under conditions of normoxia.[40]

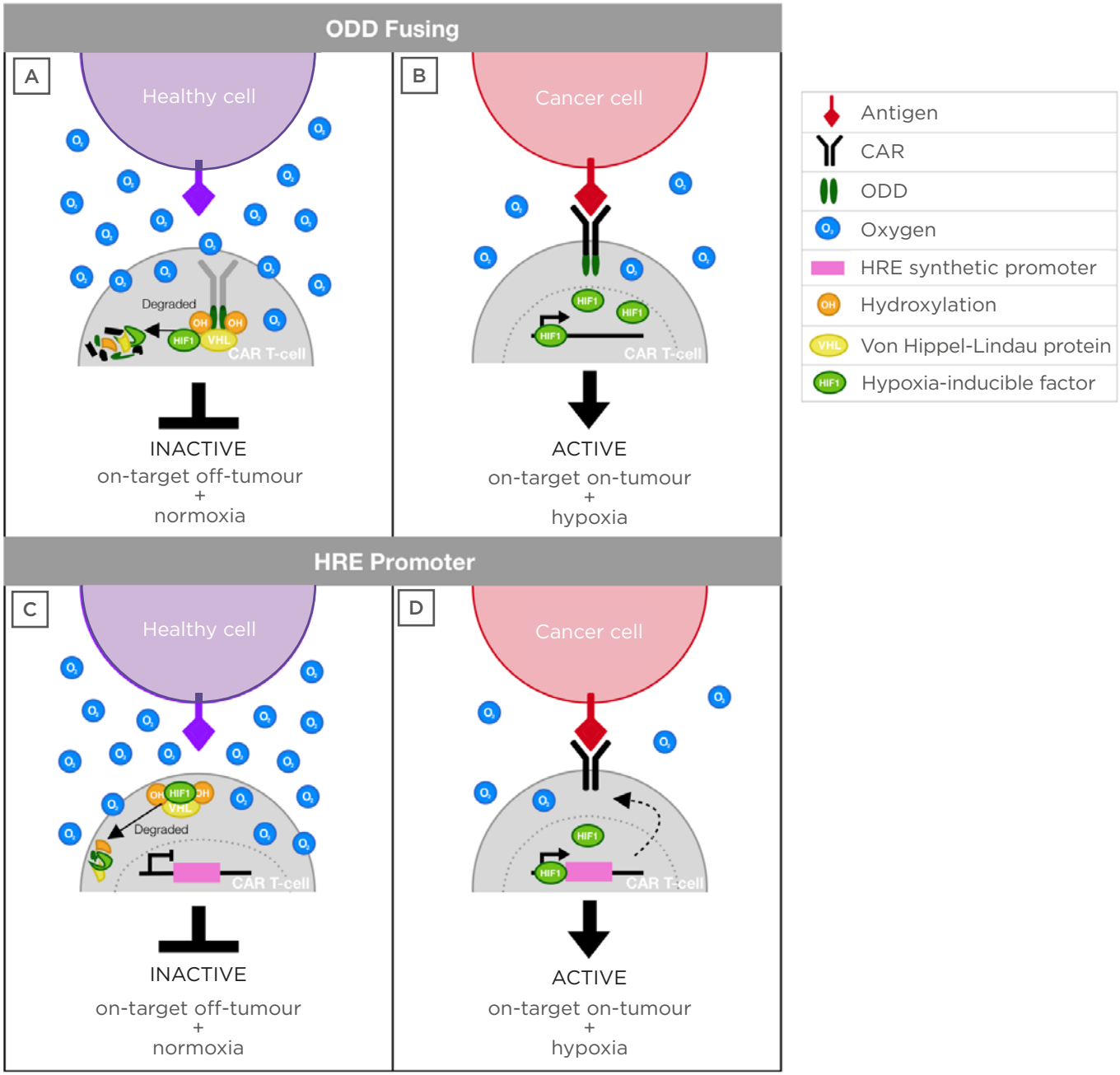

Figure 1: Schematic to illustrate hypoxia-sensing CAR-T cell approaches.

A,B) ODD fusion approach. Upon infusion, CAR-T cells may engage healthy **(A)** or cancer **(B)** cells that express cognate target. When the CAR is exposed to normoxia, the ODD fused to the CAR become hydroxylated and recognised by the VHL, which ubiquitinates the CAR for proteasomal degradation. In hypoxia **(B)**, prolyl hydroxylase does not hydroxylate the CAR, which prevents degradation and allows target-dependent CAR activation.

C,D) HRE-promoter approach. Upon infusion, CAR-T cells situated in healthy tissue **(C)** are exposed to normoxia and thus CAR transcription is not triggered, protecting healthy cells that express the antigen and avoiding on-target, off-tumour toxicity. By contrast, HIF-1 complex accumulation occurs selectively in conditions of hypoxia **(D)**, allowing the binding of this complex to HRE in the synthetic vector promoter region. As a result, CAR expression occurs, which enables the activation of these T cells upon antigen binding.

CAR: chimeric antigen receptor; HIF-1: hypoxia-inducible factor 1; HRE: hypoxia-responsive elements; ODD: oxygen-dependent degradation domain; VHL: von Hippel–Lindau protein.

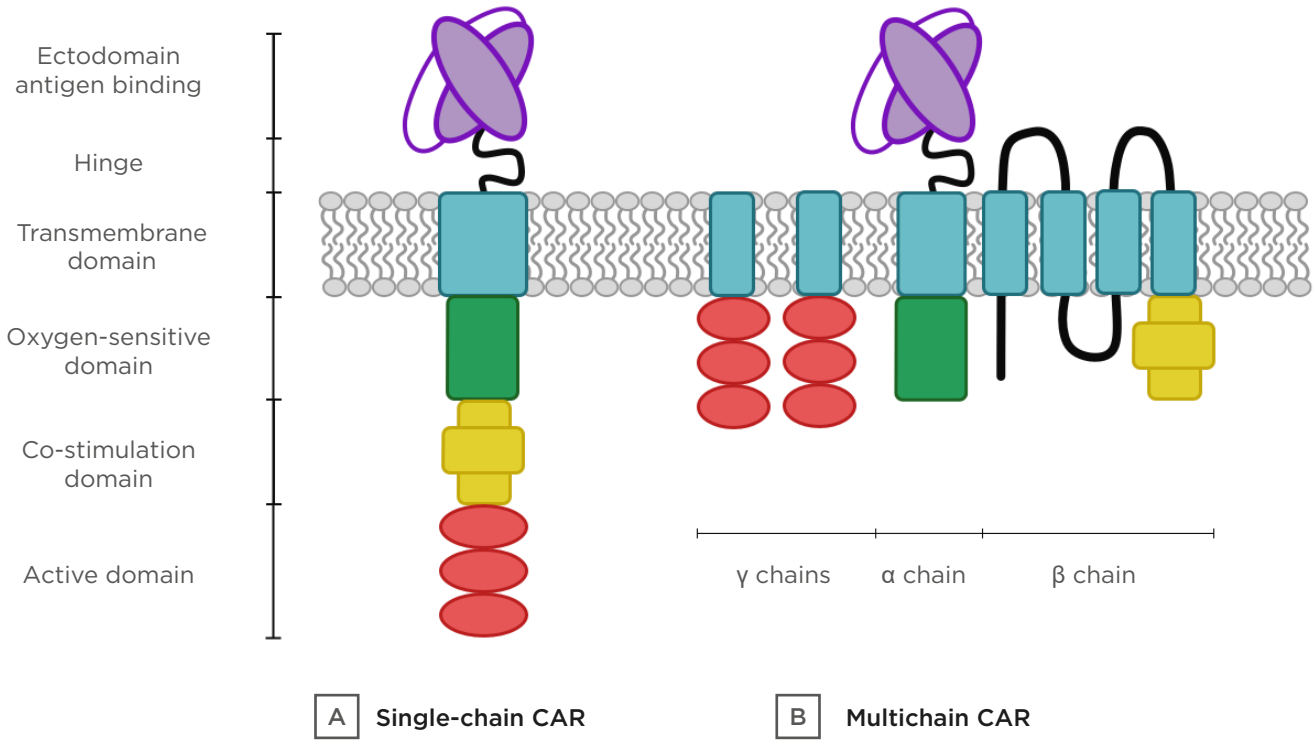

Figure 2: Multi-input CAR designs.

A) Single-chain CAR. The targeting, activation, co-stimulation, and ODD domains are fused in a unique chain.
B) Multichain CAR. The different domains are distributed in three different chains (α, β, and γ).

CAR: chimeric antigen receptor; ODD: oxygen-dependent degradation domain.

The strategies described thus far have taken advantage of the HIF master orchestrators of oxygen responsiveness, through the fusion of an ODD to the CAR endodomain (Figure 1A-B). An alternative oxygen-sensing CAR system was recently presented by Sarén et al.[45] in an abstract. They fused a cassette of hypoxia-responsive elements to a minimal cytomegalovirus promoter and evaluated the ability of this putative hypoxia-responsive promoter to drive expression of a CD19-specific CAR (Figure 1C-D). Following chemical induction of hypoxia, they showed that expression of the CAR was enhanced, whereas the expression of a control CAR under the transcriptional control of a constitutive EF1α promoter was unaltered. Additionally, the group reported that the oxygen-sensing CAR displayed enhanced activation and cytotoxic activity upon antigen recognition in conditions of hypoxia, when compared to normoxia.[45]

CONCLUSIONS AND PERSPECTIVE

The aforementioned studies provide proof of concept as to how hypoxia may be exploited to enhance precision targeting of CAR-T cell immunotherapy.

A further theoretical advantage relates to the fact that restricted CAR expression at sites of hypoxia might be expected to mitigate against the deleterious effects of tonic signalling, which is a particular problem with some single-chain variable fragment-targeted CAR.[46] Moreover, T cells that escape from the tumour microenvironment should be rapidly disarmed owing to CAR ubiquitination under oxygen-replete conditions. By this means, off-tumour on-target toxicity should be minimised. It should be acknowledged, however, that ODD-dependent CAR degradation can take between minutes and hours[47] meaning that some such toxicity might nonetheless occur.

In the further advancement of these technologies, a number of points warrant consideration. While physiological oxygen levels vary across many normal tissues, they are lower than atmospheric oxygen levels. This highlights the need for careful testing of CAR expression and function across a range of oxygen tensions. Another potential concern is that the activity of hypoxia-sensing CAR could be suboptimal in tumours with heterogeneous oxygen levels, owing to excessive CAR degradation in physiological normoxic areas.[47] Finally, leakiness of some systems could prove problematic in the clinical application of this technology. While it has been argued that low-level CAR expression under oxygen-replete conditions could help initiate CAR-T cell activation,[40] concerns remain that on-target, off-tumour toxicity could occur as a result. Additionally, inflammation triggered by low-level CAR activation might be expected to cause further local hypoxia, creating a vicious circle in which further CAR upregulation, tissue damage, and inflammation could ensue. By contrast, systems that rely exclusively on HIF-triggered CAR transcription may be undermined in part by the fact that TCR activation can also trigger HIF stabilisation via mTOR signalling.[48]

In summary, highly stringent restriction of CAR expression to the tumour microenvironment constitutes a desirable AND gate by which to restrict CAR function at the site of disease (e.g., where target and hypoxia colocalise). However, with currently available systems, this remains an unmet goal that requires further technological refinement. The successful development of such a technology would broaden extensively the range of targets and cell dose levels that could be safely employed in efforts to develop effective CAR-T cell immunotherapies for solid tumours.

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Superficial Ulcerating Rheumatoid Necrobiosis Associated with Methotrexate Use in a Patient with Rheumatoid Arthritis

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Abstract

Methotrexate, a disease-modifying antirheumatic drug, is fundamental to limiting progression in several rheumatic diseases such as rheumatoid arthritis (RA). However, methotrexate is also associated with various significant adverse effects. Of note, there are several dermatologic manifestations attributed to methotrexate therapy. In particular, accelerated nodulosis and panniculitis are linked to methotrexate therapy in the current literature. The authors present the case of a 55-year-old Caucasian female with seropositive erosive RA who developed superficial ulcerating rheumatoid necrobiosis (SURN), secondary to methotrexate therapy. The patient's treatment consisted of methotrexate discontinuation, topical clobetasol, and initiation of leflunomide as a replacement of methotrexate. Follow-up evaluation confirmed resolution of SURN over time and maintained low disease RA activity with leflunomide.

Few cases describe SURN in the setting of RA and there are currently no cases published that suggest methotrexate's possible role in SURN. Methotrexate-induced SURN is plausible in this case because of the correlation with therapy initiation and remission after therapy discontinuation. SURN has significant histological overlap with other methotrexate-induced dermatologic manifestations, allowing for a possible correlation. Most dermatological side effects of methotrexate are linked to a genetic predisposition of the *HLA-DRB1* gene. Additionally, methotrexate's mechanism of action for rheumatologic disease paradoxically stimulates adenosine-1 receptors and activates neutrophil chemotaxis and phagocytosis. Adenosine-1 receptor stimulation is hypothesised to be the source of rheumatoid-accelerated nodulosis and possibly SURN. Furthermore, the location of manifestation, genetic predisposition, and comorbid features in the patient all possibly have a role in this unique dermatological side effect.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune condition prevalent in North America and Northern European populations with an incidence of 1%.[1] It affects females disproportionately to males; the lifetime risk of development is 3.6% and 1.7%, respectively.[2] Several therapeutic regimens are available for RA under the categorisation of disease-modifying antirheumatic drugs (DMARD). Early intervention with immediate initiation of DMARD therapy is recommended to prevent radiographic progression of the disease.[3]

Methotrexate (MTX) is one of the first-line DMARD used to treat RA. Several mechanisms of action have been suggested regarding MTX efficacy as a DMARD therapy.[4] These mechanisms include inhibition of nucleotide synthesis causing precursor accumulation to a degree, but the specifics of each mechanism are unique. A proposed mechanism by Cronstein et al.[4] includes the inhibition of 5-aminoimidazole-4-carboxamide-ribonucleotide (AICAR) transformylase. This subsequently increases the concentration of AICAR, and more specifically, adenosine; adenosine then perpetuates an anti-inflammatory response.[4,5] The other, more commonly accepted mechanism is the inhibition of dihydrofolate reductase and thymidylate synthase, which induces cellular depletion and inhibition of *de novo* purine synthesis.[5]

MTX therapy has several well-documented adverse effects, with up to 20% of patients discontinuing usage in response to this.[5] Various reported toxicities of MTX are grouped into cardiotoxicity, haematologic toxicity, nephrotoxicity, pulmonary toxicity, hepatotoxicity, and carcinogenic toxicity.[5] Additionally, more common side effects that decrease patient tolerance are gastrointestinal in origin and include nausea, vomiting, and diarrhoea.[5] MTX therapy is also responsible for several dermatologic adverse effects;[6] some patients with RA beginning MTX therapy have reported accelerated nodulosis, with some cases not showing resolution after medication discontinuation.[6] This side effect has been associated with *HLA-DRB1* and activation of local adenosine receptors in genetically susceptible individuals.[6] Furthermore, additional evidence suggests a correlation between MTX-induced accelerated nodulosis and subsequent panniculitis in these susceptible individuals.[7]

Superficial ulcerating necrobiosis, a rare entity that may accompany RA, can develop to become superficial ulcerating rheumatoid necrobiosis (SURN).[8] Necrobiosis is a generalised descriptor that refers to skin without connective tissue fibres upon histological examination Histologically, these lesions are characterised by bordering palisading histiocytes, multinucleated cells, and epithelioid cells.[8] Necrobiosis is often associated with necrobiosis lipoidica diabeticorum (NLD) because these lesions demonstrate collagen degradation. Other dermatopathological manifestations may be described histologically as necrobiosis such as granuloma annulare and rheumatic nodules.[8] Physical examination of skin lesions may reveal a shallow ulcer formation, typically found on the legs; although, erythematous papular eruptions without ulceration have been reported.[9] MTX-induced SURN has not been described in the current literature and warrants further discussion.

The authors herein present the case of a 55-year-old Caucasian female with seropositive erosive RA who developed SURN after initiation of MTX therapy.

CASE DESCRIPTION

A 55-year-old female with 20-year history of seropositive (rheumatoid factor and anti-CCP positive) erosive RA on MTX therapy presented with firm, yellow/violaceous, depressed papules and plaques with central telangiectasias on the bilateral shins, calves, and dorsal feet. Some lesions had raised edges, and sizes ranged from 5 mm to 3 cm (Figure 1). Her eruption had started upon initiation of MTX therapy longer than 10 years ago, and she reported increased size and number of plaques in subsequent years. She had chosen not to pursue evaluation for the rash priorly as it had been asymptomatic, but it was now becoming increasingly disfiguring. She had had low RA disease activity for the previous few years with a Clinical Disease Activity Index (CDAI score ranging from 2–5 and Disease Activity Score-28 and C-reactive protein (DAS-28-CRP) scores ranging from 1.63–1.93 over the past year on MTX 25 mg

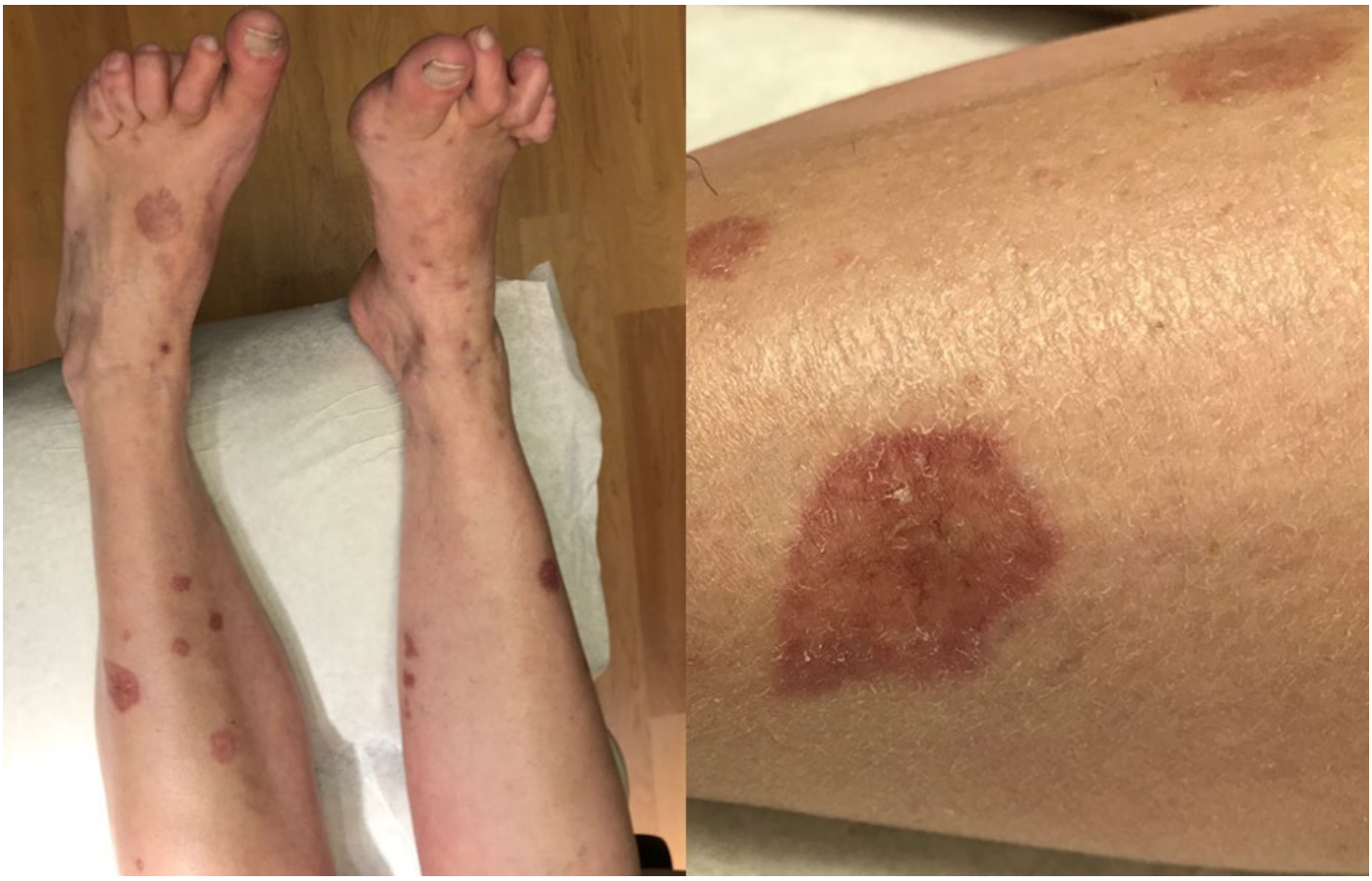

Figure 1: Initial presentation with firm, yellow/violaceous, depressed papules and plaques with central telangiectasias on the bilateral shins, calves, and dorsal feet. Some lesions presented with raised edges and sizes ranging from 5 mm to 3 cm.

subcutaneous weekly monotherapy. She did not have any subcutaneous rheumatoid nodules.

A skin biopsy demonstrated dermal palisading granulomas with central necrobiotic collagen (Figure 2). There were several associated lymphocytes, plasma cells, neutrophils, and eosinophils. Periodic acid–Schiff and acid-fast bacilli stains were negative for infectious organisms. A colloidal iron stain did not demonstrate increased dermal mucin (Figure 2). No vasculitis was identified on the histopathology. These findings were thought to be compatible with palisading granulomatous dermatitis with necrobiotic collagen, consistent with SURN.

Given the historical suggestion by the patient about the onset of the eruption coinciding with initiation of MTX, her MTX was discontinued and she was started on leflunomide to treat her RA. She was also started on topical clobetasol 0.05% ointment. After 2 months, the eruption significantly improved, with all old lesions fading and no presentation of new lesions. Topical corticosteroids were discontinued and on 6-month follow-up the eruption had resolved, with some residual post-inflammatory hyperpigmentation despite topical corticosteroid cream discontinuation several months previously (Figure 3). The patient maintained low RA disease activity on leflunomide.

SURN has been reported in patients with RA, but the authors found no reported cases of MTX-associated SURN in the literature. Despite this, MTX-induced accelerated nodulosis is a well-reported phenomenon. Given the strong timeline association of rash onset after initiation of MTX therapy and the improvement in rash after discontinuing MTX, it is highly probable that the SURN was caused by methotrexate in this patient.

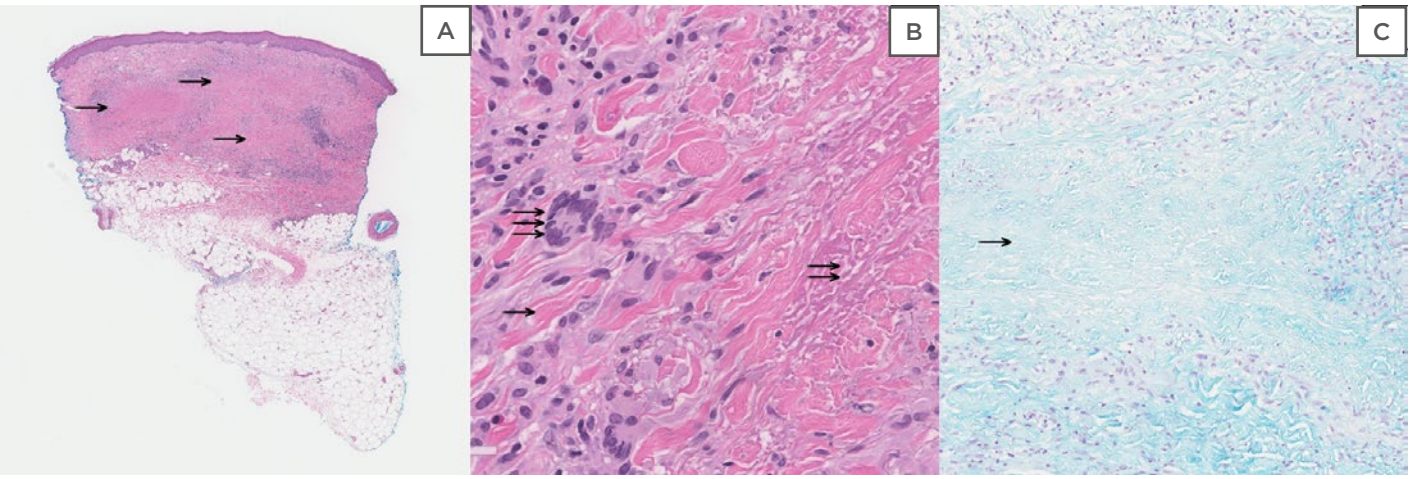

Figure 2: A) 4 mm punch biopsy of the skin; haematoxylin and eosin stain (low power, 2x magnification) demonstrates palisaded granulomatous dermal inflammation (single arrows). **B)** Haematoxylin and eosin stain (high power, 40x magnification) demonstrates intact peripheral collagen bundles (single arrow), necrobiotic collagen (double arrow), and surrounding palisaded granulomatous dermal inflammation with multinucleated histiocytes (triple arrow). **C)** Colloidal iron stain (intermediate power, 10x magnification) is negative for increased dermal mucin in zones of necrobiotic collagen (single arrow).

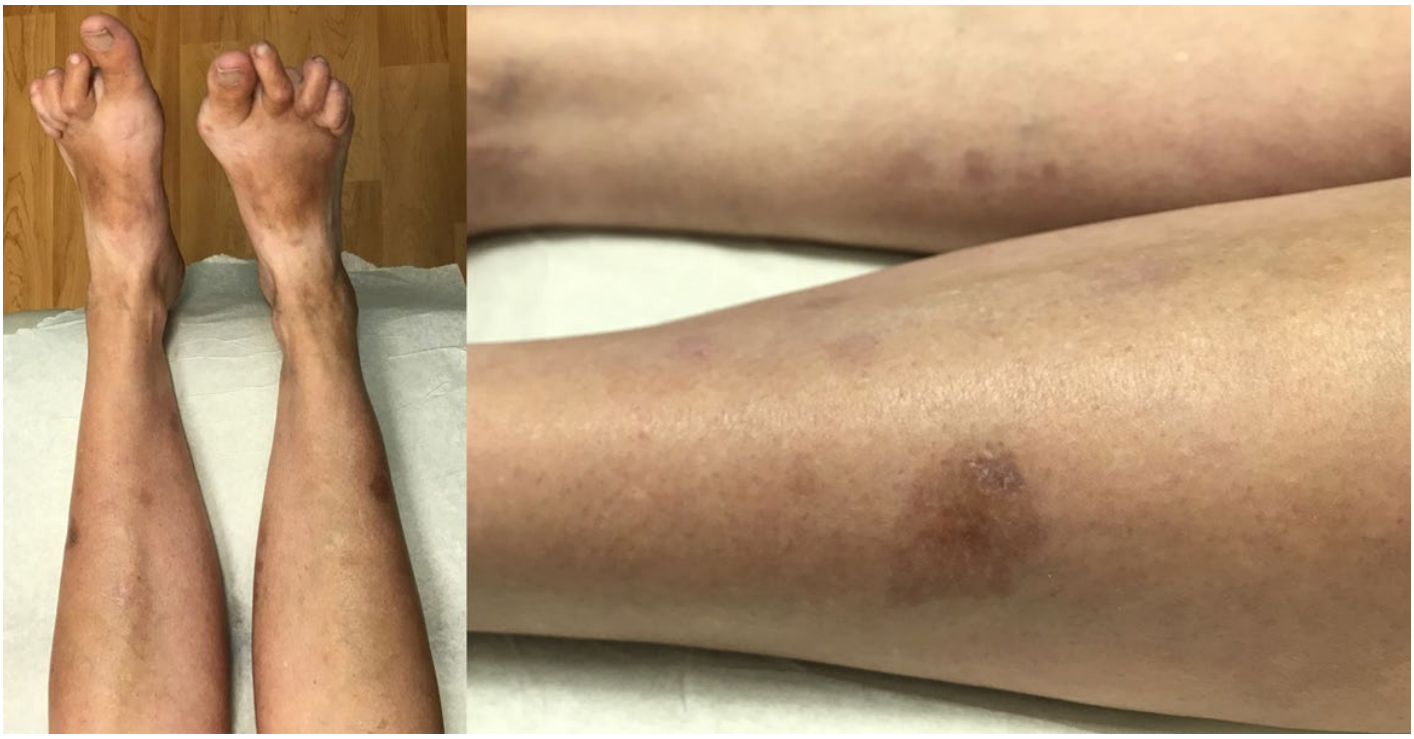

Figure 3: Resolution of eruption is shown, with residual post-hyperpigmentation changes 6 months after discontinuing methotrexate.

DISCUSSION

MTX is a widely used DMARD and is often the first-line treatment for RA. MTX has been well known to cause accelerated nodulosis in patients with RA and there are some reports showing the prevalence of nodulosis is as high as 8% amongst patients treated solely on MTX.[10] Additionally, accelerated nodulosis induced by MTX has been reported in patients with duration of therapy ranging from 3 months to 12 years after treatment initiation.[11] Therefore, the role of MTX is plausible in this described case.

Several extra-articular cutaneous manifestations of RA have levels of overlap based on histology. Generalised necrobiosis is evident through focal degeneration of connective tissue and necrobiosis can be found in several other pathologies including rheumatoid nodules, granuloma annulare, and NLD.[12] Histology analysis shows disrupted collagen fibres with collagen hyalinisation. The mechanism of necrobiosis is linked to immune complex deposition, which leads to vasculitis and subsequent tissue remodelling by matrix metalloproteinases (MMP).[12]

Rheumatoid nodules present in RA can show evidence of necrobiosis in their histological description. Classically, they are subcutaneous, firm, mobile nodules found predominately on, but not limited to, extensor surfaces of the body.[13] Rheumatoid nodules are classified histologically into three distinct parts, as described by Yamamoto et al:[13] the first descriptor is a core of necrosis with eosinophilic infiltrate and the second and third histologic findings include a palisading zone and chronic inflammatory cells infiltrating the perivascular space.[13] Cells that primarily predominate in the palisading zones include activated macrophages and T-cell lymphocytes. These cells are thought to be responsible for the necrobiosis.[14] Necrobiosis and the subsequent breakdown of Type 1 collagen is thought to be secondary to TNF-α cytokine-induced activation of MMP-1 and MMP-3.[15] Ulceration is rarely described in the setting of rheumatoid nodules but could be plausible in patients with comorbid vascular insufficiency.[13]

SURN is rarely described in RA. Jorizzo et al.[8] describe two cases of patients who presented with leg lesions similar to the patient described in this report. Both cases had aggressive RA and developed focal ulcerating lesions in the lower legs; however, both patients' lesions were classified as NLD.[16] This seemed unlikely because these patients had rheumatoid nodules, mild rheumatoid vasculitis, and high-titre rheumatoid factor levels. Therefore, the authors proposed a distinct entity of patients with a histologically similar process related to subclinical vasculitis and immune complex deposition.[16]

Using information gathered from similar, overlapping skin manifestations in RA, it is possible to deduce a pathway for further exploration of MTX-induced SURN. MTX-induced accelerated nodulosis also has some overlap with the above described patient's presentation in terms of both histology and history. Accelerated nodulosis has been documented across a variety of dosages and durations of treatment.[11] Furthermore, it has a histological presentation of palisading granulomas and focal necrobiosis similar to the above described case.[13] Since MTX-induced accelerated nodulosis and panniculitis are linked to patients expressing *HLA-DRB1*, it is important to note there is a certain genetic susceptibility present in these patients.[6,7] Therefore, the hypothesised mechanism of MTX's role in the pathogenesis of SURN could be similar.

MTX has been hypothesised to induce rheumatoid nodules through its mechanism of action. Understanding this mechanism helps isolate the potential cause behind the acceleration of rheumatoid nodules. As previously mentioned, the therapeutic mechanism of action for MTX as DMARD therapy is through the inhibition of AICAR transformylase.[4] This causes an increase of AICAR, which inhibits conversion of adenosine monophosphate to inosine monophosphate.[15] Additionally, AICAR increases adenosine to excessive levels. This stimulates low affinity adenosine-2 receptors located on inflammatory cells like neutrophils, monocytes, lymphocytes, and basophils, halting synthesis of cytokines and other inflammatory molecules.[4] Side-effect manifestation is linked to increased extracellular adenosine-stimulating high-affinity adenosine-1 (A1) receptors.[4,13] A1 receptors are present on macrophages and neutrophils and on these white blood cells they enhance immunologic functionality through

heightened chemotaxis and phagocytosis of immunoglobulins.[4,13] While histological examination for IgM was not investigated in this case, past reports have shown evidence of IgM deposition in dermal blood vessels with both SURN and rheumatoid nodules.[8,13] Activated macrophages and neutrophils through A1 receptors may then more readily phagocytose IgM complexes within dermal blood vessels. This is the likely mechanism behind DMARD therapy and is the possible manifestation of accelerated nodulosis or SURN.[13]

While correlative data and overlapping disease characteristics may help support the notion of SURN development from MTX therapy, direct causality could not be attributed between manifestation and pharmacologic agent. This case review is limited by the lack of a re-challenge with MTX. Re-introducing MTX would allow observation for the redevelopment of SURN. Reformation of SURN after a re-challenge would provide significant strength behind the hypothesis that the dermatologic manifestation was secondary to drug therapy. While a re-challenge analysis would be beneficial, subjecting a patient to possible redevelopment of the disease would be unethical in the clinical setting. In addition, re-challenging one patient in this study would not provide the statistical power necessary to consider the possible harms of evaluation. Future investigation and the details of this report will allow future examiners to assemble a larger sample size and appropriately develop a more substantial re-challenge assay.

CONCLUSION

In the few cases describing SURN, the cases presented as shallow ulcers on the lower legs, commonly the pretibial area. These cases had significant overlap with NLD and were often considered as such.[8] It is hypothesised that these lesions are formed from a combination of factors including leg location, possible presence of vasculitis, and possible comorbid vascular insufficiency.[13] These factors, combined with MTX-stimulating inflammation or necrobiosis in a genetically susceptible individual via A1 receptors could be the plausible pathophysiology responsible for the described case. Nevertheless, further cases and research would help elucidate the possible role of MTX in the formation of SURN.

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Economic Evaluation of Severe Anaemia: Review-Based Recommendations and a Conceptual Framework

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Abstract

Objective: Novel anaemia treatments have greatly improved patient outcomes in the last decade and have also undergone economic evaluations in various settings using heterogenous model structures, costs, and inputs. The objectives were to review published economic evaluation studies in major red blood cell disorders, identify limitations in the applied methodology, provide a set of recommendations, and produce a conceptual framework for future economic research in this disease area.

Methods: A targeted search was conducted for economic literature evaluating treatments in major red blood cell disorders related to anaemia. Disorders included autoimmune haemolytic anaemia, β-thalassaemia, chemotherapy-induced anaemia, anaemia in chronic kidney disease, and severe aplastic anaemia. Budget impact models and cost-effectiveness and cost-utility analyses were considered. Modelling assumptions regarding the model structure, time horizon, perspective, and type of costs were reviewed and recommendations and a conceptual framework for future economic analyses were created.

Results: A total of four budget impact models, nine cost-utility analyses, and four cost-effectiveness analyses were investigated. A major limitation was that the included costs varied significantly across studies. Costs which were rarely included, and generally should be considered, were related to adverse events, mortality, and productivity. Additionally, relationships between levels of serum ferritin, hepatic or total body iron, and haemoglobin with long-term complications and mortality were rarely included.

Conclusion: Published economic analyses evaluating treatments for major red blood cell disorders frequently exclude vital costs. A set of recommendations and a conceptual framework will aid researchers in applying a more comprehensive approach for economic evaluations in major red blood cell disorders.

INTRODUCTION

Anaemia is characterised by a decrease in the total amount of red blood cells (RBC) and haemoglobin (Hb) levels, reducing the volume of oxygen transported through the blood.[1] Several varieties of anaemia exist, and the disease pathology can be explained either as a disease-related complication (e.g., anaemia of inflammation) or as a general hereditary condition.[2,3] Anaemia classifications and conditions leading to severe anaemia include autoimmune haemolytic anaemia (AIHA), β-thalassaemia, chemotherapy-induced anaemia, anaemia in chronic kidney disease (CKD), and severe aplastic anaemia (SAA); many of which are associated with early mortality.

RBC transfusions were frequently used as a standard of care in the treatment of anaemia; however, regular RBC transfusions are accompanied with serious health risks.[4] Iron overloading, related to regular RBC transfusions, can lead to fatal complications including organ failure. One class of alternative therapies, erythropoiesis-stimulating agents (ESA), received U.S. Food and Drug Administration (FDA) approval in 1989 for anaemia related to chronic renal failure.[5] Subsequently, a combination of ESA and RBC transfusions are generally used to treat severe anaemias. Recently, new treatment strategies have been undergoing clinical development with several novel therapies gaining regulatory approval. Novel therapies are also accompanied with high costs, which can act as potential barriers to patient access. Economic evaluations aid decision makers to assess whether the reimbursement of novel therapies would be an efficient use of limited healthcare resources and if they can be afforded with current healthcare budgets.

The purpose of this review is to identify relevant literature and explore the budgetary impacts of novel treatments for severe anaemia and whether they were found to be cost-effective. The benefit of reviewing economic literature is two-fold. Firstly, standardised cost-effectiveness analyses (CAE) in severe anaemias will allow clinicians to consider lifetime clinical outcomes, which may not be adequately captured in clinical trials, the length of which is generally limited to 1–2 years. Reviewing standardised economic evaluations would help clinicians consider long-term health implications in their decision-making process. Secondly, as novel therapies are generally very costly, it is important for clinicians to consider whether the added clinical benefit of a therapy justifies the incremental cost in settings with limited healthcare resources. Standardised economic evaluations in this disease area will allow clinicians to compare various treatments across analyses and aid decision-making while considering budgetary implications.

To the authors' knowledge, no manuscript investigating the evolution of severe anaemia treatment from an economic perspective has been published. As severe anaemia is generally a chronic disease, careful economic modelling should be employed to appropriately capture long-term disease implications.

METHODS

A targeted literature review was conducted via PubMed Central (PMC), Google Scholar, and OVID. The disease area of interest included any type of severe anaemia evaluated in a published comprehensive economic analysis. Although stem cell transplant treatments are important in this disease area, they are significantly different from ongoing, continuous therapies, which are the focus of this analysis. Differences such as cost structures and cure potential result in different designs and, for this reason, stem cell transplants are excluded from this analysis. No other limits on the type of treatments were employed. Search terms included a combination of a disease term with a study design term. Disease terms included "anemia", "beta-thalassemia", "autoimmune hemolytic anemia", "chemotherapy-induced anemia", "sickle cell disease", "chronic kidney disease anemia", and "severe aplastic anemia". Study design terms included "cost-effectiveness analysis","cost-utilityanalysis",and"budgetimpact model". Any economic outcome was included. Additional limits included studies published in English and studies published between 2010 and 2020. Subsequently, the methodology of identified studies was critically assessed. Recommendations for future economic evaluations were developed based on a critical assessment. Additionally, a conceptual framework was created to aid future economic analyses,

building on past studies' strengths, and identifying gaps to be avoided in future research.

RESULTS

Overview

A total of 18 studies were identified which met the inclusion/exclusion criteria. The following elements were explored for each study: disease area, study design and perspective, treatments, time horizon, costs, measures of uncertainty, as well as the model structure and health states. A summary of studies is presented in Table 1.[6-23]

Diseases

Seven studies evaluated therapy for β-thalassaemia, one for AIHA, one for chemotherapy-induced anaemia, one for chronic heart failure-related anaemia, two for SAA, and six for CKD-related anaemia.

Study Design and Perspective

Four studies were budget impact models (BIM), four were CEA, and 10 were cost-utility analyses (CUA). From the 18 studies identified, two were Canadian, three were from the USA, two from Australia, two from Italy, and one from the UK, Thailand, China, Iran, Poland, Germany, South Korea, and Morocco, respectively. A total of 16 studies used a healthcare system perspective while two others used a societal perspective.

Table 1: Identified publications.

Disease	Country	Study	Type of study	Time horizon	Perspective	Treatment
β-thalassaemia	UK	Bentley et al.,[6] 2013	CUA	5 years	Healthcare payer	DFO, DFP, DFX, combination therapy (DFO plus DFP)
	Thailand	Luangasanatip et al.,[7] 2011	CUA	Lifetime	Societal	DFX versus DFP
	Australia	Karnon et al.,[8] 2012	CUA	50 years	Healthcare payer	DFX versus DFO
	China, Taiwan	Ho et al.,[9] 2013	CUA	50 years	Healthcare payer	DFX versus DFO
	Iran	Keshtkaran et al.,[10] 2013	CUA	Lifetime	Societal	DFX versus DFO
	Poland	Walczak et al.,[11] 2013	CUA	1 year	Healthcare payer	DFX versus DFO
	Italy	Pepe et al.,[12] 2017	CUA	5 years	Healthcare payer	DFX versus DFP
Autoimmune haemolytic anaemia	Italy	Rognoni et al.,[13] 2018	BIM	3 and 5 years	Hospital and taxpayer	Rituximab originator versus rituximab biosimilars and SC versus IV
Chemotherapy-induced anaemia	Greece	Nikolaidi et al.,[14] 2013	BIM	15 weeks	Social security funds	ESA originator versus ESA biosimilar

Table 1 continued.

Disease	Country	Study	Type of study	Time horizon	Perspective	Treatment
Chronic heart failure with iron deficiency anaemia	South Korea	Lim et al.,[15] 2015	CUA	24 weeks	Healthcare payer	Ferric carboxymaltose versus placebo
Severe aplastic anaemia	USA	Tremblay et al.,[16] 2019	BIM	3 years	Private healthcare system	EPAG plus ATGAM plus cyclosporine versus ATGAM plus cyclosporine
	Germany	Heublein et al.,[17] 2013	CEA	1 year	Healthcare payer	h-GAM (ATGAM) versus R-GAM (Thymoglobulin® [Sanofi, Paris, France])
	USA	Yarnoff et al.,[18] 2016	CEA	Lifetime	Healthcare payer	ESA for optimal Hb level
Chronic kidney disease-related anaemia	Australia	Wong et al.,[19] 2013	CEA	Lifetime	Healthcare payer	IV versus oral iron supplementation
	Canada	Clement et al.,[20] 2014	CUA	Lifetime	Healthcare payer	ESA versus without ESA
	Canada	Tsao et al.,[21] 2014	BIM	5 years	Healthcare payer	ESA originator versus ESA biosimilar
	Morocco	Maoujoud et al.,[22] 2016	CUA	1 year	Healthcare payer	Continuous erythropoietin receptor activator versus epoetin beta versus RBC transfusion
	USA	Quon et al.,[23] 2012	CEA	5 years	Healthcare payer	ESA for optimal Hb level

BIM: budget impact model; CEA: cost-effectiveness analysis; CUA: cost-utility analysis; DFX: deferasirox; DFP: deferiprone; DFO: desferrioxiamine; EPAG: eltrombopag; ESA: erythropoiesis-stimulating agent; Hb: haemoglobin; IST: immunosuppressive therapy (ATGAM plus cyclosporine); IV: intravenous; NHS: National Health Service; RBC: red blood cell; SC: subcutaneous.

Treatments

For β-thalassaemia, the study treatments assessed were various chelation therapies (deferoxamine, deferiprone, deferasirox, and combination of deferoxamine plus deferiprone).

The AIHA study compared rituximab against a biosimilar, also comparing subcutaneous versus intravenous administration. For chemotherapy-induced anaemia, ESA originators (Aranesp® [Amgen, Thousand Oaks, California, USA] [darbepoetin alpha], NeoRecormon [Roche, Basel, Switzerland] [epoetin beta], and Eprex [Janssen, Beerse, Belgium] [epoetin alpha]) were compared to biosimilars (Abseamed [MEDICE, Iserlohn, Germany] [epoetin alpha], Binocrit® [Sandoz, Holzkirchen, Germany] [epoetin alpha], and Retacrit® [Pfizer, New York City, New York, USA] [epoetin zeta]). For SAA, two identified studies assessed eltrombopag and ATGAM® (Pfizer), respectively, against immunosuppressive therapy. For CKD-related anaemia, treatments

were related to the usage of ESA for patients on or without dialysis. For the chronic heart failure-related iron-deficient anaemia, iron supplementation was evaluated against placebo.

Time Horizon

Time horizons identified across studies ranged from 15 weeks to a lifetime. Shorter time horizons (15 weeks to 5 years) were employed in ten studies (56%) while two used 50 year-horizons (11%), and a lifetime horizon was used in six (33%). As chemotherapy-induced anaemia is a non-chronic condition, a 15-week horizon was applied. Excluding chemotherapy-induced anaemia, and chronic heart failure-related anaemia, time horizons ranged from 1 year to a lifetime.

Costs

All studies included drug costs (100%), 12 studies included administration costs (67%), and seven studies included healthcare resource utilisation costs (39%). Indirect costs were frequently missing from analyses, specifically, adverse events, mortality, and productivity costs. They were included in five (28%), two (11%), and one (6%) studies, respectively. Administration costs were excluded in two biosimilar studies and biosimilar studies also excluded indirect costs, which were assumed to be similar for both treatment arms.

Uncertainty

A total of 15 studies (83%) included sensitivity analyses, which assessed stochastic and deterministic uncertainty by varying inputs and testing model assumptions. Two BIM and one CUA did not report any assessment of uncertainty.

Model Structure and Health States

The model structure varied greatly across different studies. For CEA and CUA, the model structure was reported as Markov models for nine studies (50%), microsimulations for two studies (11%), and unreported for three studies (17%). Four additional studies were BIM (24%). The number of health states varied from three to seven across diseases.

DISCUSSION

Overview and Conceptual Framework

Economic evaluations in severe anaemia were reviewed with the objective of creating recommendations for individual components of future economic analyses. The lack of economic-focussed guidelines for severe anaemia demonstrates a gap in the literature. Conceptual frameworks have been developed in multiple other therapeutic areas including immune thrombocytopenia[24] and acute coronary syndrome.[25] As anaemia is generally a chronic condition with long-term implications, it is vital to consider if short-term trial endpoints can accurately be extrapolated to long-term horizons and outcomes. Moreover, structural heterogeneity across economic evaluations can hinder cross-study comparisons. A validated conceptual economic framework used in future research could facilitate easier comparisons. As long-term complications are associated with severe anaemia and standard of care treatments, relevant biomarkers such as levels of Hb and serum ferritin should be included as proxies to quantify the long-term risk of complications.

A general conceptual framework was developed for CEA and CUA in severe anaemia. It is possible that different disease-specific considerations and economic questions would require a tailoring of the conceptual framework. However, the one presented in Figure 1 has been designed to accurately capture the implications of chronic anaemia and related RBC transfusions, including long-term complications related to iron overloading (serum ferritin proxy) and reduced or elevated Hb, treatment-related adverse events, key costs, key efficacy measures, and important endpoints. The conceptual framework may help clinicians and formulary committees evaluate the robustness of economic analyses. This, in turn, could help formulary committees consider which treatments obtain formulary coverage and subsequently affect the clinician's ability to prescribe these treatments. Additionally, standardised economic study designs could help clinicians evaluate the long-term effectiveness of therapies across different analyses and aid clinical decision-making.

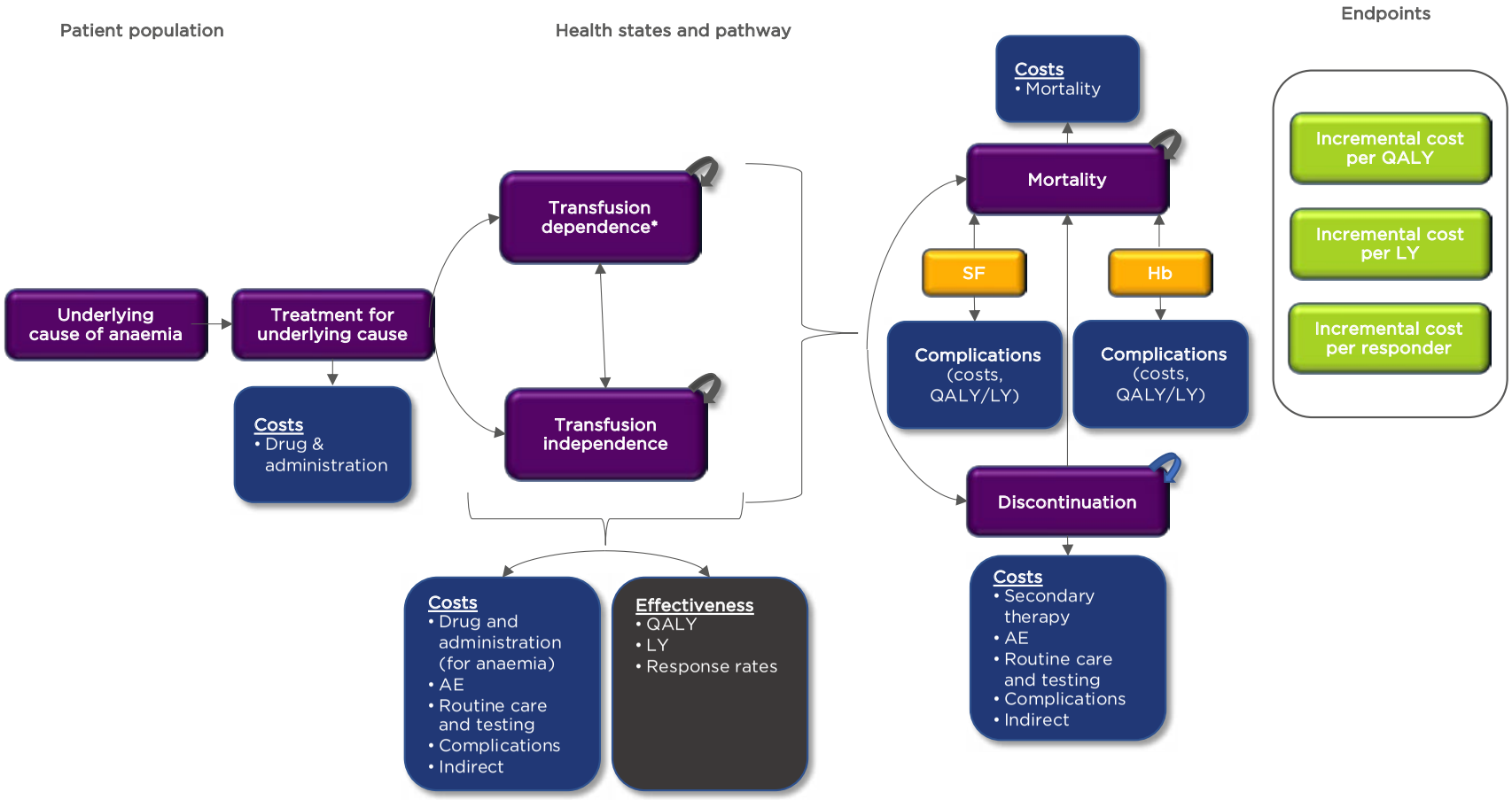

Figure 1: Conceptual framework for cost-effectiveness analysis and cost-utility analysis evaluations of severe anaemia disorders.

*Transfusion-dependent health states should be separated by level of transfusion burden when possible (if data permits). This will facilitate a more granular, accurate approach as different levels of transfusion burden area are associated with different costs and quality of life.

Colour legend: purple: patient pathway and various health states; blue: costs (and effectiveness as well for complications); grey: measures of effectiveness; yellow: biomarkers which should be included in the model; green: economic evaluation endpoints.

AE: adverse event; Hb: haemoglobin; LY: life year; QALY: quality-adjusted life year; SF: serum ferritin.

Additional components requiring further consideration are summarised in the following subsections.

Treatment Comparisons

As economic evaluations are comparative by nature, it is important to specify which techniques or trial designs were used to compare treatments and to report the source of data. Typically, trial-based data should be utilised as a source of clinical inputs for novel therapies. In cases where multiple trials are used as data sources, an indirect treatment comparison (ITC) should be considered. Naïve, unadjusted ITC could lead to bias because of differing baseline patient characteristics and trial heterogeneity. If the baseline characteristics between treatment arms are imbalanced, an ITC should be conducted. Appropriate ITC require individual patient data which is matched for baseline characteristics to another trial population using statistical techniques. Two ITC methods are recommended, including the simulated treatment comparison and the matching-adjusted indirect comparison, whose objectives are to produce a covariate-adjusted treatment effect estimate. Propensity scoring and regression form the basis of their methods and both are recommended by the National Institute for Health and Care Excellence (NICE).[26] One important consideration is to assess whether trial data can accurately be extrapolated to long-term outcomes. As this can vary with different types of severe anaemia and trial design, it is recommended to consult a clinical expert.[27]

Health States

Transfusion-dependent patients have a higher likelihood of experiencing complications than their non-dependent counterparts, potentially necessitating a greater number of hospitalisations and outpatient visits.[28] Iron overload related to RBC transfusions is associated with long-term cardiac and hepatic infection and endocrine complications.[29] Multiple studies found an association between quality of life and Hb levels in transfusion-dependent patients.[30-32] Additionally, numerous studies found that complications related to iron overloading, estimated using serum ferritin, were also associated with reductions in quality of life and utility.[33-35] Therefore, levels of serum ferritin and Hb should be accounted for throughout the horizon of the model with elevated levels of serum ferritin and reduced/elevated levels of Hb leading to complications and quality of life decrements. One study showed that patients with lower (9.0–10.9 g/dL) and higher (>12.0 g/dL) Hb levels have increased frequencies of adverse events and early mortality.[20] These differences could be reflected in different health states. If the data permits, multiple health states with various levels of transfusion burden, serum ferritin, and hepatic or total body iron count should be used to account for higher frequency of RBC transfusions and related long-term complications. The evaluated diseases are mostly chronic by nature and long-term horizons should be prioritised. Models should also incorporate the dynamic nature of complications and patient burden, which increase with time.[36]

Costs

As anaemia is a chronic condition, costs which arise over an extended period should be considered. Both direct and indirect costs should be included when comparing two different treatments. Several key costs were absent from the literature. Adverse events, long-term complications, mortality, and productivity costs should be included to create a comprehensive analysis. The proposed conceptual framework enables the inclusion of numerous costs including the cost to treat the underlying cause of anaemia (e.g., β-thalassaemia), which can vary between transfusion burden, health states, and anaemia severity. A granular approach between transfusion burden health states (e.g., high versus low) is preferable if data is available, because the annual costs of RBC transfusions can vary with frequency of administration. Anaemia severity may be evaluated through iron concentration or Hb levels, but is economically manifested through the cost of RBC transfusions and other supporting therapies (e.g., iron chelation therapy), adverse events, long-term complications, routine care and testing, early mortality, and indirect costs.

Complications and Adverse Events

Serum ferritin is used to assess iron levels and oxygen levels are used to estimate Hb. Depending on anaemia severity, patients may require

frequent RBC transfusions, resulting in long-term iron overloading, long-term complications, and treatment-related adverse events.[37] Examples of anaemia-related long-term complications and treatment-related adverse events include cardiovascular disease, cerebrovascular disease, and infection.[38] Complications and adverse events could lead to elevated costs through more frequent hospitalisations, outpatient visits, and potentially early mortality. Levels of Hb were only directly considered in five economic evaluations (29%). A modelling approach which includes dynamic rates for the risk of long-term complications based on transfusion burden and Hb and serum ferritin levels is recommended. A gap in the published analyses was identified as no economic studies considered the relationship between complications and serum ferritin levels.

In four identified studies, adverse events affected quality of life; however, their costs were not considered. It is recommended that economic evaluations include both cost and quality of life effects for long-term complications and adverse events.

Sensitivity Analysis

All economic evaluations should contain an uncertainty assessment. It is essential to test assumptions and quantify the stochastic effect/probabilistic and deterministic uncertainty on the results of the analyses. Evaluations without uncertainty assessments are not comprehensive. For BIM, it is recommended to perform one-way deterministic sensitivity analyses which varies inputs individually in various scenarios. The magnitude of variance should be obtained from published literature or from consultation with disease-specific clinical experts.[39] For CEA and CUA, one-way deterministic and probabilistic sensitivity analyses are also recommended. In probabilistic sensitivity analyses, all inputs should be varied simultaneously for 1,000–10,000 iterations to identify a range of incremental cost-effectiveness ratios and to estimate the probability that an incremental cost-effectiveness ratio falls under a willingness-to-pay threshold. The stochasticity of variables is assessed using their standard error and a randomly generated value across specific distributions. Appropriate distributions should be selected based on variable characteristics. Generally, the gamma distribution should be considered for costs, beta or gamma distributions for utility, and log-normal distributions if a skew to the right is needed. Uncertainty analysis is vital to assess robustness. NICE guidelines also recommend exploring the impact of uncertainty on the results of the economic analyses.[40]

Limitations

The proposed framework and set of recommendations were specifically designed for economic evaluations related to severe anaemia. It should be noted that disease-specific anaemias, e.g., CKD-related anaemia, could require modellers to adapt and tailor the framework by adding dialysis or kidney transplant pathways or health states, for example. The disease-specific framework may also require further adjustments based on the availability of data for the disease, for example, the availability of an association between health states and mortality. Additionally, although ITC is recommended using individual patient data, it is plausible that future modellers may have limited access to this type of data, potentially impeding the implementation of this recommendation. No economic evaluations were identified for several common anaemias including sickle cell anaemia. Notably, the Institute for Clinical and Economic Review (ICER) also released an evidence report on sickle cell disease in March 2020. The report states that sickle cell disease has been an underfunded area for research; therefore, there is limited literature on the subject.[41] Additionally, it should be noted that, while the analysis is focussed on severe anaemia or conditions related to severe anaemias, anaemia may not be the sole determinant of costs and quality of life. The underlying disease would also have a significant effect. Several treatments targeting anaemia, such as iron chelation therapy, would however, require economic evaluations.

An additional limitation is regarding the validity of serum ferritin as a proxy for iron overload. Other methods, such as T2*-weighted MRI of the liver or other major organs, may capture iron overload more accurately.[42,43] However, as clinical trials would frequently be the source of inputs for economic models, their relatively short durations may not allow variations of iron concentration from T2*-weighted MRI to correlate with patient quality of life, an essential

consideration in cost-effectiveness analysis. Serum ferritin levels fluctuate more rapidly relative to T2*-weighted MRI. Another reason serum ferritin is frequently used to assess the level of iron overload is its relatively low cost and ease of implementation compared with other techniques.[44] Weekly or monthly T2*-weighted MRI is unlikely to be recommended, as fluctuations require longer periods of time. MRI is recommended every 6 to 24 months depending on the condition severity; this testing interval can be longer than typical clinical trials.[45]

CONCLUSION

The targeted literature review demonstrated numerous gaps in published economic evaluations for severe anaemia-related diseases. By identifying these gaps, a conceptual framework and set of recommendations were created for future economic evaluations in severe anaemia-related diseases. Key considerations for future analyses consist of the inclusion of Hb and iron concentration levels in the model, multiple transfusion-dependent health states (if possible), long-term complications, adverse events, early mortality, and productivity costs. Future economic analyses which use a consistent anaemia-specific framework will allow decision makers to appropriately compare various published studies, critique the robustness of their analyses and ultimately aid them in their reimbursement decision-making process to optimise the use of scarce healthcare resources.

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Metastasis: A Bane of Breast Cancer Therapy

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Abstract

The underlying mechanisms of metastasis in patients with breast cancer is still poorly understood. Approximately 6% of patients with breast cancer present with metastasis at the time of diagnosis. Metastatic breast cancer is difficult to treat and patients with breast cancer with distant metastasis have a significantly lower 5-year survival rate compared to patients with localised breast cancer (27% and 99%, respectively). During breast cancer progression, tumour cells first metastasise to nearby draining lymph nodes and then to distant organs, primarily bone, lungs, liver, and brain. In this brief review, the authors discuss breast cancer metastasis, the role of epithelial–mesenchymal transition and the contributions of the immune system to the metastatic process. The authors also briefly discuss whether there is any relationship between tumour size and metastatic potential, and recent advances in treatment for metastatic breast cancer. The studies highlighted suggest that immunotherapy may play a more significant role in future patient care for metastatic breast cancer.

INTRODUCTION

Breast Cancer: From Detection to Treatment

Breast cancer is one of the most commonly diagnosed cancers among women.[1] Despite advances in treatment for breast cancer, approximately 10% of women currently fail primary management strategies for early breast cancer and go on to develop recurrence, ultimately succumbing to the disease within 5 years. For those who present with late stage breast cancer, the odds of dying within that same timeframe increases to 30%.[2] Furthermore, 6–10% of women diagnosed with breast cancer

have Stage IV, or metastatic, breast cancer that has spread to other organs, primarily the lung, liver, brain or bone; 85% of breast cancer deaths are attributable to metastasis.[2] Although several subtypes of breast cancer have now been identified, breast cancers are often categorised into four major molecular subtypes: luminal A and luminal B, based on oestrogen receptor (ER) and progesterone receptor (PR) status; human epidermal growth factor receptor 2 (HER2)-positive; and basal-like or triple-negative breast cancers (TNBC), which are ER-, PR-, and HER2-negative.[3]

Detection of breast cancer can be through self-detection but, most often, breast cancers are identified by mammographic screening[4] followed by breast biopsy.[5] Prolactin-inducible protein, mammaglobin, and GATA-3 are used to determine whether an unknown metastatic cancer is of breast origin.[6] Additionally, there are blood-based assays which detect breast cancer-associated biomarkers such as carcinoembryonic antigen (CEA).[7] Treatment for breast cancer primarily comprises surgical intervention, followed by endocrine (hormonal) therapy, chemotherapy, radiation therapy, or some combination of these therapeutic approaches.[8] Endocrine therapy is recommended in patients with breast cancer with hormone (oestrogen and progesterone) receptor-positive (HR-positive) tumours.[8] The menopausal status of the patient plays a major role in the decision to recommend a particular therapeutic strategy.[9] While tamoxifen is the drug of choice for premenopausal patients,[9] aromatase inhibitors (such as letrozole) are preferred for postmenopausal patients.[10] For patients with HR-negative breast tumours, chemotherapy is the treatment of choice and is often recommended in patients with TNBC and HER2-positive breast cancer.[11] Radiation therapy may also be used to further shrink breast tumours after surgical intervention.[12]

Patients with early-stage and localised breast cancer generally have a good prognosis with approximately 99% of patients surviving over a 5-year period.[1] However, a recent study showed that at the cessation of 5 years of endocrine therapy, the risk of breast cancer recurrence remained relatively significant even after 15 to 20 years.[13] This observation suggests that 5 years of treatment is not sufficient and that a longer period of therapy should be considered to prevent breast cancer recurrence. In this review, the authors give a brief overview of the existing challenges metastatic breast cancer presents and highlight some recent advances in treatment strategies.

BREAST CANCER METASTASIS: FROM PRIMARY TUMOUR TO DISTAL SITES

Metastasis is a series of events that involve the invasion of the basement membrane at the primary tumour site, movement of the tumour cells into circulation, and subsequent dissemination to other organs.[14] During metastasis, cells lose their epithelial nature and assume a mesenchymal phenotype, and extracellular matrix proteins in the basement membrane are degraded by proteolytic enzymes, such as matrix metalloproteinases.[14] Collectively, these processes allow the cancer cells to leave the primary tumour site and escape into the circulation (blood or lymphatics) in a process known as intravasation. Generally, breast cancer first spreads to lymph nodes in close proximity to the breast through the lymphatic system, while metastasis to distant organs usually occurs via the blood.[15] Once in circulation, the breast cancer cells, now referred to as circulating tumour cells (CTC),[16] migrate to different organs in the body. CTC are often scarce in non-metastatic breast cancer but higher in advanced breast cancer disease.[17] Some markers used for CTC detection include the epithelial cell adhesion molecule, an epithelial cell marker. However, cancers can alter their expression of these markers; for instance, by undergoing epithelial–mesenchymal transition (EMT).[18] CTC are now being utilised for the early detection of breast cancer[19] and the evaluation of metastatic risk in patients.[17] However, there are controversies concerning the detection of CTC in the blood because of the absence of accurate and specific markers.[18] Alternatively, other biomarkers, such as exosomes, may also be used for determining diagnosis or prognosis, or to predict response to therapy.[20] When CTC move out of the blood to metastatic sites in a process known as extravasation,[21] they are then referred to as disseminated tumour cells.[16] At these metastatic sites, the disseminated tumour cells form new tumour foci and spread throughout the affected organ, ultimately contributing to

patient mortality. It is believed that metastasis involves an interplay between cancer cells and the metastatic tissue or organ, as some cancer cells are able to better form metastasis than others and some metastatic organs are more receptive to cancer cells than others.[22] The metastatic process is reviewed by Lambert et al.[14]

Epithelial–Mesenchymal Transition

EMT is the process by which epithelial cells acquire a mesenchymal phenotype during cancer development.[23] EMT is induced by key transcription factors such as Snail, Slug, Twist, and ZEB1[14] and is characterised by loss of cell–cell adhesion, apical–basal polarity, and expression of epithelial markers such as E-cadherin and cytokeratin, while mesenchymal markers, including N-cadherin and vimentin, are upregulated.[24] This shift from an epithelial to a mesenchymal phenotype enables the cancer cells to detach from the primary tumour, invade the basement membrane, and migrate to a distant organ and establish there.[25] Following transition, the cancer cells then acquire the ability to initiate tumour formation, which is a typical feature of cancer stem cells.[26] This tumour-initiating ability is a critical step in the metastatic process as it ensures that the disseminated breast cancer cells are capable of forming tumours at metastatic sites.[14] Across different breast cancer subtypes, TGF-β signalling has been shown to be important for EMT and is potentially a master regulator of this process in breast cancer cells. This has been reviewed by Singh et al.[27]

The Immune Response

The immune system plays a critical role in the progression of many cancers including breast cancer. Similar to certain components of the immune system that are known to participate in the prevention and elimination of cancer, other components of the immune system can promote cancer progression and metastasis.[28] Myeloid-derived suppressor cells (MDSC), alternatively activated (M2) macrophages, and regulatory T cells have been shown to secrete factors that suppress anti-tumour immune responses.[29] MDSC secrete TGF-β and vascular endothelial growth factor (VEGF) which stimulate EMT and angiogenesis, facilitating tumour growth and metastasis.[30] MDSC also release the anti-inflammatory cytokine IL-10: a potent suppressor of anti-tumour immune responses.[30] Additionally, macrophages in the tumour microenvironment have been shown to display the M2 phenotype; M2 macrophages are important for wound healing and tissue repair.[31] Like MDSC, M2 macrophages release TGF-β, IL-10, and chemokines which promote breast cancer growth and metastasis.[32] In addition to suppressing anti-tumour cytotoxic and helper T cells via cell-to-cell contact, regulatory T cells also secrete IL-10 which dampens anti-tumour immune responses.[33] Studies also indicate that neutrophils, another group of immune cells, can enhance metastasis by inhibiting cytotoxic T cell function,[34] secreting leukotrienes, and promoting the proliferation of tumour-initiating cells at the metastatic environment.[35]

Does Tumour Size Matter?

Although previous studies suggested that the relationship between tumour size and metastasis was linear, recent data from a large population study of more than 800,000 patients with breast cancer showed that it is not.[36] This study showed that at the time of diagnosis, a patient with a tumour 7 cm in diameter has an approximately equal chance of having lymph node metastasis compared to a patient with a 15 cm tumour (71.8% versus 71.3%, respectively).[37] The discrepancy between previous studies and the current study may be attributed to the small sample size and inaccurate methods of those previous studies. In addition to axillary lymph node metastasis, studies also show that larger tumour sizes correlated with worse clinical outcomes.[38] However, many patients with TNBC, especially the subset that express basal markers such as cytokeratin, do not follow this trend.[36] Indeed, smaller basal tumours have been associated with more lymph node metastasis than non-basal tumours and had worse disease outcomes than expected; larger basal tumours were associated with better outcomes than expected.[39] Most of the mutations found in the primary tumour of a patient with basal-like breast cancer were also found in metastatic cells of the patient, suggesting that the primary tumour cells already had the necessary features needed to metastasise.[40] Other parameters such as biomarker expression, e.g., Ki67, were found to correlate with both lymph node metastasis and

tumour size.[41] It appears that size is not the only determining factor for metastatic spread; the subtype and intrinsic biology of the tumour may play critical roles as well.

PREFERRED SITES FOR BREAST CANCER METASTASIS

The draining, or sentinel, lymph node in the axilla, which is located in close proximity to the breast tumour site, is usually the first site of metastasis.[42] Breast cancer then spreads to distal sites, most frequently bone, lungs, liver, and the brain.[43] Estimates from a recent study showed that at the time of diagnosis, patients with metastatic breast cancer were found to most frequently present with bone metastasis (3.28%), followed by lung (1.52%) and liver (1.20%) metastasis, while the brain was the least common site for metastasis (0.35%).[44]

Metastasis to Bone

Current understanding suggests that metastatic breast cancer cells are able to take advantage of the natural remodelling process in the bone to facilitate metastasis by disrupting the balance between formation and resorption of bone, leading to bone loss (osteolysis) or abnormal bone formation.[45] One process for causing this disruption is by secreting factors to modulate their environment.[46] Breast cancer cells have been reported to secrete IL-11 and matrix metalloproteinases which can stimulate osteoclasts to produce growth factors that, in turn, promote the growth and survival of the metastatic breast cancer cells in the bone.[46] Interestingly, results from recent studies also suggest that prolactin overexpression in breast tumours can shorten the time to development of bone metastasis and contribute to osteolysis induced by the metastatic breast cancer cells.[47] Among all breast cancer subtypes, patients with luminal breast cancer have the highest rate of bone metastasis.[48]

Metastasis to the Lungs

Breast cancer cells released from the primary tumour may also spread to the lungs.[49] Lung metastasis is usually observed 5 years after initial breast cancer diagnosis, with >60% mortality rate following lung metastasis.[49] The subtype of breast cancer affects the frequency of lung metastasis. Patients with TNBC were reported to be more likely to develop lung metastasis compared to other subtypes.[48] The development of lung metastasis in patients with breast cancer may be because of an inherent ability of breast cancer cells to the lungs, or an ability to interact with the lung microenvironment. Premalignant breast cells have been shown to invade the lungs after injection into the blood, suggesting that breast cancer cells may have some intrinsic ability to establish in the lungs.[50] It has been shown *in vitro* that the migration and proliferation of breast cancer cells were stimulated by the conditioned medium of the lungs, suggesting that factors from the lungs promote lung metastasis. One such factor identified was selectin, a cell adhesion molecule.[51]

Metastasis to the Liver

Another common site for breast cancer metastasis is the liver. Liver metastasis leads to impairment of liver function with serious outcomes, and without treatment intervention life expectancy is <8 months.[52] Patients with HER2-positive breast cancers show the highest incidence of liver metastasis.[48]

Metastasis to the Brain

Although less common, breast cancer can also metastasise to the brain. Patients with breast cancer and brain metastasis have poor prognosis, display neurological defects, and do not respond well to therapy.[53] Due to more successful treatment of the primary tumour, patients with breast cancer live longer, and brain metastasis more frequently becomes evident later in the disease process.[54] The propensity of breast cancer to metastasise to the brain seems to depend on the breast cancer subtype.[55] Brain metastasis occurs in approximately 50% of patients with TNBC, and 33% and 14% of patients with HER2-positive and HR-positive breast cancer, respectively.[48]

ADVANCES IN TREATMENT MODALITIES FOR METASTATIC BREAST CANCER

Standard Therapeutic Drugs

The treatment strategies utilised for patients with metastatic breast cancer depend on several

factors. These factors are often based on two considerations: the breast cancer subtype and the stage of the disease. Generally, patients with HR-positive breast cancer are treated with endocrine therapy, patients with HER2-positive breast cancer are treated with anti-HER2 antibody therapies, such as trastuzumab, while triple-negative tumours are typically treated with chemotherapy.[3] The initial treatment regimen for patients with metastatic HR-positive breast cancer includes endocrine therapy in combination with a cyclin-dependent kinase (CDK) 4/6 inhibitor, such as abemaciclib. Patients with resistance to endocrine therapy are treated with chemotherapy.[3] For patients with HER2-positive breast cancer, the first line of treatment includes a combination of an anti-HER2 antibody therapy, such as trastuzumab, and a chemotherapy, e.g., a taxane. If this initial treatment is unsuccessful, trastuzumab emtansine, an antibody–drug conjugate, is then administered.[3] If this treatment also fails, continuous use of the HER2-targeted therapy may be recommended, in combination with a different chemotherapeutic agent. Unlike HR- and HER2-positive breast cancers, TNBC are only treated with chemotherapy because of a lack of established molecular targets, as reviewed by Waks et al.[3]

New Therapeutic Drugs

Several new therapeutic agents have been recently approved by the U.S. Food and Drug Administration (FDA), while a number are still undergoing clinical trials. Sacituzumab govitecan is an antibody–drug conjugate, currently undergoing testing in clinical trials, which has shown positive effects in previously treated patients with advanced TNBC.[56] Another promising drug is atezolizumab, an inhibitor of programmed death-ligand 1, and nanoparticle albumin-bound-paclitaxel, which was shown to enhance progression-free survival in patients with breast cancer.[57] To treat patients with metastatic breast tumours harbouring the *BRCA1/2* mutations, poly(ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, were recently approved by the FDA.[58] PARP inhibitors are used to counteract the DNA repair brought about by PARP, to enhance the sensitivity of cancer cells to DNA-damaging anti-cancer therapies, and ultimately lead to cell death.[59] However, many of these drugs are only successful in extending the patients' lives for a short period of time. Response rates are often poor, and many patients develop serious side effects. A study reported that among histological subtypes, patients with invasive lobular carcinoma had increased progression-free survival compared to patients with invasive ductal carcinoma; however, there was no difference in overall survival.[60] Across molecular subtypes, patients with HR-positive/HER2-positive breast tumours had better survival outcomes, while those with TNBC had worse outcomes.[61] Another challenge is that following systemic therapy, a population of dormant breast cancer cells may persist and subsequently lead to relapse.[14] This situation is further complicated by the cytotoxic treatments used against breast cancer mainly targeting highly proliferating cells with minimal effects on dormant cells.[14]

Immunotherapy: Homing in on our Immune Response

Immunotherapy is a treatment strategy that harnesses the ability of the immune system to keep diseases, including cancers, at bay. In recent years, there have been some successes in using immunotherapy to treat certain type of cancers such as melanoma, which is considered to be an immunogenic cancer, capable of eliciting an immune response.[62] Breast cancer was previously not considered to be an immunogenic cancer, but it has now been shown that the presence of immune cells in some breast cancers is of prognostic and therapeutic significance.[62] Breast cancers with greater influx of tumour-infiltrating lymphocytes tend to respond better to neoadjuvant chemotherapy compared to those with fewer tumour-infiltrating lymphocytes.[29]

Recently, some immunotherapeutic approaches have been assessed for the treatment of a limited number of patients with breast cancer. These include the use of tumour antigen-specific antibodies and adoptive transfer of activated immune cells, such as natural killer cells, dendritic cells, and T cells.[28] A recent study was the first to demonstrate no signs of cancer in a patient with metastatic breast cancer following treatment by adoptive transfer of activated patient-derived tumour-specific T cells.[63] Pembrolizumab, an anti-

PD1 antibody, has been shown to be effective for patients with metastatic TNBC in clinical trials.[64] Vaccines are also being explored as another treatment strategy. The use of recombinant HER2 protein in patients with advanced HER2-positive breast cancer was shown to induce an anti-tumour immune response and benefit a limited number of patients.[65] The use of chimeric antigen receptor T cells is another immunotherapeutic strategy currently being developed for treatment of breast cancer. Studies using chimeric antigen receptor T cells engineered to target the mucin 1 protein expressed on breast cancer cells have shown promising preclinical results[66] and this therapy is currently undergoing clinical trials.[67] Major challenges with these approaches include the cost of treatment and the difficulty in isolating sufficient activated tumour-specific T cells from patients. However, efforts are being made to reduce these challenges through the utility of immune cells from unrelated donors without tissue rejection.[68] Some current therapeutic approaches are summarised in Table 1.

CONCLUSION

The wide degree of heterogeneity that exists among breast cancers presents many challenges for both clinicians and patients. There are still significant gaps in knowledge about the metastatic process during breast cancer progression, including: why different breast cancer subtypes preferentially metastasise to specific organs; why tumour size does not always matter; and why some breast cancers trigger an immune response while others do not. Advances in the understanding of the interaction between several cancers and the immune system could lead to the use of immunotherapy as a viable option for the treatment of some patients with breast cancer with metastatic disease. There is also a need for greater understanding of key signalling pathways. However, a multidisciplinary approach to research is warranted to address these gaps in knowledge and to develop effective strategies to benefit a large number of patients. Furthermore, current comprehensive guidelines for the treatment of metastatic breast cancer provided by key European and American societies, such as the European Society for Medical Oncology (ESMO)[69] and the National Comprehensive Cancer Network (NCCN),[70] are largely similar in their recommendations. However, some discrepancies do exist, highlighting that further research is warranted to fully determine which therapies are most effective and beneficial for patients in the long term.

Table 1: Examples of current therapeutic approaches for metastatic breast cancer patients.

Treatment	Breast cancer subtype	Overall survival
Standard therapies		
Endocrine therapy only, or in combination with CDK 4/6 inhibitor (abemaciclib) Endocrine therapy and chemotherapy (taxane)	HR-positive breast cancer	4–5 years[3]
Anti-HER2 antibody (trastuzumab) and taxane Trastuzumab emtansine	HER2-positive breast cancer	5 years[3]
Chemotherapy (taxane, anthracycline)	TNBC	10–13 months[3]
New therapies		
Sacituzumab govitecan	TNBC	13 months[56]
Atezolizumab and nanoparticle albumin-bound paclitaxel	TNBC	25 months (patients with PD-L1-positive tumours)[57]
Pembrolizumab	TNBC	9 months[61]

CDK: cyclin-dependent kinase; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; PD-L1: programmed death-ligand 1; TNBC: triple-negative breast cancer.

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Sjögren's Syndrome Complicated with Type 2 Autoimmune Hepatitis: A Case Report-Based Review of the Literature

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Abstract

This case details a 33-year-old female who presented with a suggestive autoimmune history, arthralgias, and splenomegaly, and tested positive for Sjögren's syndrome and anti-liver–kidney microsomal antibody. This was further validated by findings from a liver biopsy, confirming a very rare association with Type 2 autoimmune hepatitis. Primary Sjögren's syndrome is a sporadic disease with a global prevalence of 61 per 100,000 people and a total prevalence of 0.4% for secondary Sjögren's syndrome. The prevalence of autoimmune hepatitis in association with primary Sjögren's syndrome is 4–47%. It is divided into two types, associated with characteristic antibodies. Type 2 autoimmune hepatitis is rarely reported with Sjögren's syndrome; much of the association reported in the literature has been with Type 1 autoimmune hepatitis.

INTRODUCTION

Autoimmune hepatitis is a condition closely related to autoimmune diseases. Previously, it was known to have three distinct types, which were recently further refined into two distinct types, each associated with characteristic antibodies. Sjögren's syndrome is an autoimmune disease[1] infrequently associated with autoimmune hepatitis; when it is, there is an association with the Type 1 subset of antibodies, such as antinuclear antibody (ANA), anti-smooth muscle antibody, perinuclear antineutrophil cytoplasmic antibodies, or antimitochondrial antibody, which usually is associated with primary biliary cirrhosis.[2] Type 2 autoimmune hepatitis is associated with the anti-liver–kidney microsomal (LKM) antibody. This case presented with a suggestive history of Sjögren's syndrome and additional workup revealed massive splenomegaly, which was further characterised as a feature of autoimmune hepatitis supported by the serological testing. An enlarged spleen could have multiple causes

and implications to be ruled out in this present case, including myeloproliferative disorders, and Leishmaniasis, HIV, and Cytomegalovirus infections. The unique feature about this case was an association with Type 2 autoimmune hepatitis, which is overall less prevalent than Type 1 autoimmune hepatitis,[3] and the association of Type 2 autoimmune hepatitis with Sjögren's syndrome was a very rare entity in the literature review.

CASE PRESENTATION

A 33-year-old female of Asian ethnicity presented, without prior known comorbidities, with arthralgias, low-grade fever, stinging, foreign body sensation in eyes, and dry mouth for a long time. The patient denied photosensitivity, oral ulcers, alopecia, or history of abortions. Along with these symptoms, the patient had unintentional weight loss, anorexia, and abdominal discomfort preceded by abdominal fullness. She had no history of drug allergies or blood transfusion and was not taking any medications or alcohol. The examination was unremarkable, except for mild pallor along with hepatosplenomegaly. The patient was initially managed with low-dose intravenous antipyretics for the fever, oral nutritional supplements, and was given artificial tears for eye symptoms.

Laboratory workup showed a haemoglobin of 7.7mg/dL, mean corpuscular volume of 82 fL, total leukocyte count of 2.2 cells/µL with differentials of 61% neutrophils and 27% lymphocytes, and platelet count of 49 x10^9/µL of blood. It also showed increased erythrocyte sedimentation rate (83 mm/hour); elevated transaminases (normal range: <45 IU/L), aspartate aminotransferase: 213 IU/L; alanine aminotransferase: 239 IU/L; total bilirubin: 0.8 mg/dL; γ-glutamyltransferase: 20 IU/L; alkaline phosphatase: 165 IU/L; normal albumin: 3.7 g/dL, serum creatinine: 0.9 mg/dL; and prothrombin time: 12.8 sec. Hypergammaglobulinaemia was noted and there were elevated serum IgG levels of 2,712 mg/dL. Serology for viral infections, HBsAg, anti-hepatitis C (HCV), hepatitis B core antibody IgM, HIV, and Cytomegalovirus, was negative. Further workup showed positive ANA with titres of 2+ (granular), negative double-stranded-DNA, rheumatoid factor of 81 U/mL, strongly positive anti-Sjögren's syndrome-A antibodies with titres of 41 U/mL, anti-Sjögren's syndrome-B antibodies with titres of 14 U/mL, and anti-LKM was positive on two occasions with the presence of adequate negative and positive controls for immunofluorescence.

Ultrasonography of the abdomen was consistent with the above-mentioned examination findings; hence, CT of the abdomen was carried out to rule out intrahepatic vascular or thrombotic changes and was negative except for massive splenomegaly of 21.7 cm (Figure 1). The next aim was to obtain a liver biopsy, which demonstrated lymphoplasmacytic infiltrate with prominent plasma cells in the portal tracts with marked interface activity and multiple areas of hepatic necrosis consistent with autoimmune hepatitis (Figure 2). The sonographic findings of major salivary glands were remarkable for multiple hypoechoic lesions with high vascularity, thereby supporting the diagnosis of Sjögren's syndrome in association with autoimmune hepatitis. Rose Bengal staining by ophthalmology remains the confirmatory diagnostic test of Sjögren's; but, no objective measurements of oral and ocular dryness were performed. The biopsy of the labial glands was deferred by the patient.

The patient was started on prednisolone (60 mg/day), according to the patient's weight, for 1 month and azathioprine (50 mg/day) after 2 weeks of treatment initiation. The response was monitored by improving cytopenias, the gradual return of liver enzymes to normal limits, and decreased IgG levels. After 2 months of treatment, steroids were tapered off on a follow-up visit and the patient was kept on azathioprine (100 mg/day) for a further 6 months. Laboratory workup at follow-up showed haemoglobin of 9.6 mg/dL, mean corpuscular volume of 84 fL, total leukocyte count of 4.5 cells/µL with differentials of 67% neutrophils and 24% lymphocytes, platelet count of 146 x 10^9 /µL of blood, aspartate aminotransferase 43 IU/L, and alanine aminotransferase 39 IU/L, with no fever documented.

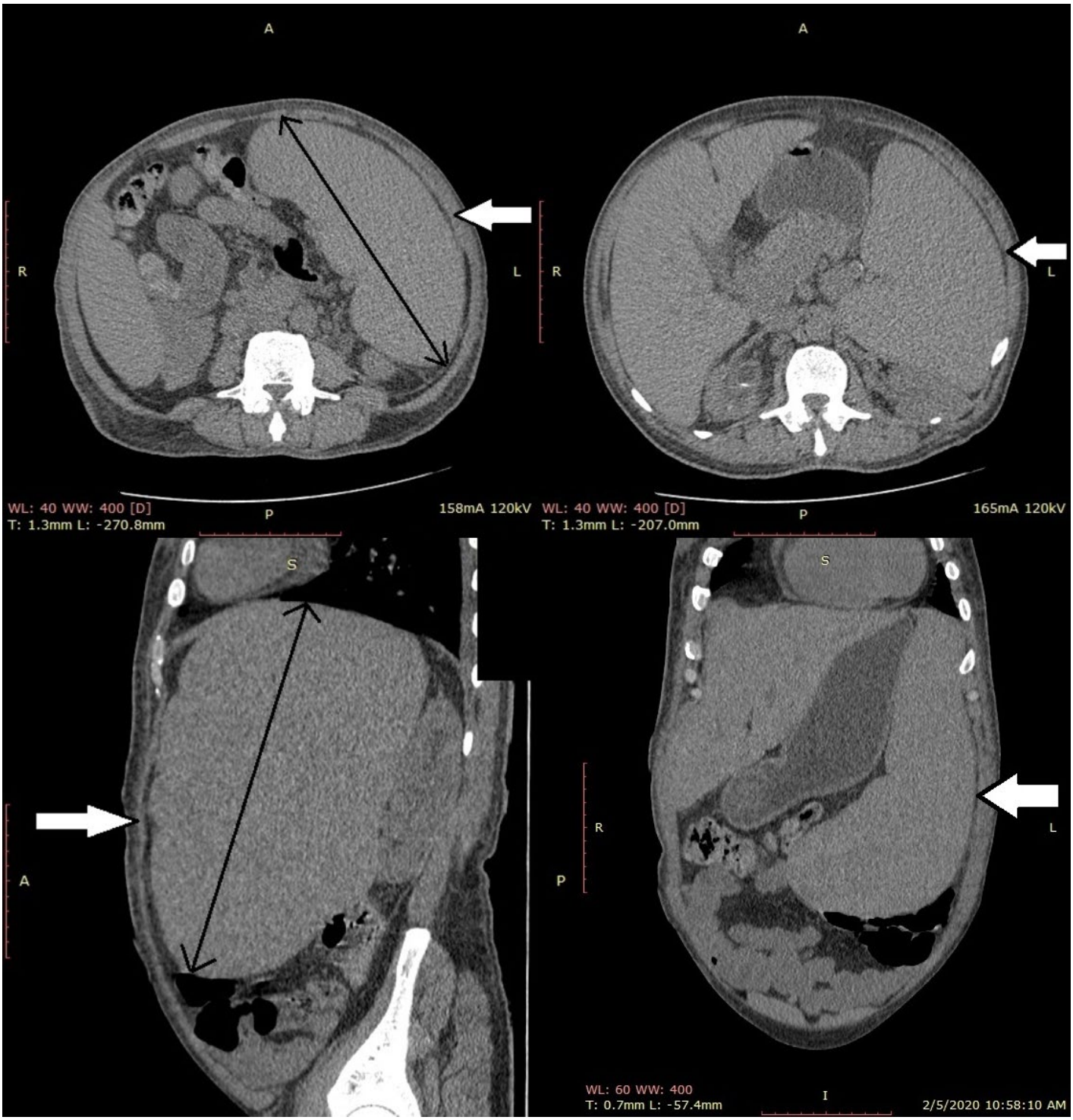

Figure 1: Axial, coronal, and sagittal sections of an abdominal CT scan showing splenomegaly.

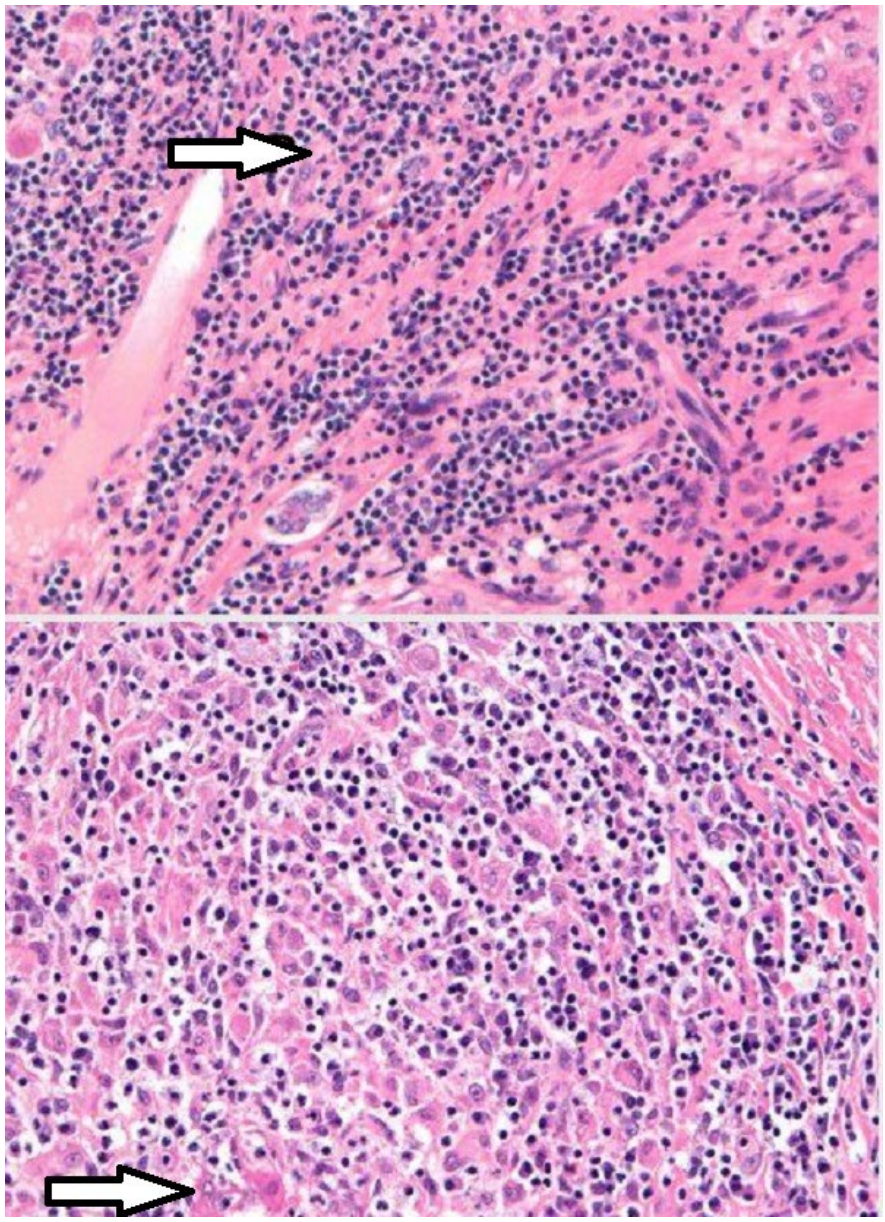

Figure 2: Histopathology showing lymphoplasmacytic infiltrate with marked interface hepatic necrosis.

DISCUSSION

Primary Sjögren's syndrome is a sporadic disease with a global prevalence of 61 per 100,000 people with peak incidence in Europe and a total prevalence of 0.4% for primary and secondary Sjögren's syndrome.[1] Sjögren's syndrome is an autoimmune disease affecting many systems in the body, resulting in lymphocytic infiltration of salivary and lacrimal glands, progressing towards exocrine failure and fibrosis. Sjögren's syndrome is categorised by the primary and secondary syndrome relating to autoimmune diseases including systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, primary biliary cirrhosis, and autoimmune hepatitis.[1-4] The most encountered clinical manifestations of Sjögren's syndrome are keratoconjunctivitis sicca, xerostomia, salivary gland hypertrophy, rashes and skin oversensitivity, vaginal dryness with dyspareunia, arthralgias, Raynaud's phenomenon, generalised osteoarthritis, and exhaustion. Less prevalent manifestations include low spiking fever, anaemia, leukopenia, vasculitis, thrombocytopenia, interstitial lung disease, cryoglobulinaemia, peripheral neuropathy, and glomerulonephritis.[5] The predicted age of disease onset is 40–50 years, and most patients

are females.[1] Sjögren's syndrome also targets nonexocrine organs such as the lungs, thyroid, kidney, liver, and pancreas, and the central nervous system.[2,6,7]

The American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) criteria are widely accepted for the diagnosis of Sjögren's syndrome. They are defined as a score of ≥4 in relation to diagnostic features. This is along with labial salivary gland biopsy displaying lymphocytic infiltrates, positive anti-Sjögren's-syndrome-related antigen, also known as anti-Ro, antibodies, an ocular staining score of >5 in at least one eye, positive Schirmer's test with <5 mm in a span of 5 min, and salivary flow rate <0.1 mL/min with dry eyes and mouth. These criteria apply to any patient with at least one symptom of ocular or oral dryness. Conditions excluded from criteria are those with sarcoidosis, active HCV infection, amyloidosis, acquired immunodeficiency syndrome, IgG4-associated disease, graft-versus-host disease, and with radiotherapy of the head and neck.[3,7,8] Treatment modalities for Sjögren's syndrome include tear and saliva replacements, cevimeline, cyclosporine, pilocarpine, corticosteroid eye drops, and topical fluoride.[6,9] Factors prognostic of distressing outcomes in Sjögren's syndrome include declining levels of C4 complement, purpura, and mixed monoclonal cryoglobulinaemia.[5] The hepatic manifestation of Sjögren's syndrome includes primary biliary cirrhosis, HCV, nonalcoholic fatty liver disease, and autoimmune hepatitis.[2,6,10]

Autoimmune hepatitis is an unsettling and advancing disease of unexplained aetiology comprised of hypergammaglobulinaemia, autoantibodies, and hepatitis. It is characterised by the gradual disintegration of liver parenchyma.[2,11,12] Autoimmune hepatitis is prevalent predominantly in females and often reactive to immunosuppressive modalities of treatment.[2,3,12] It is associated with human leukocyte antigen (DR-3 and -4).[11,13] The prevalence of autoimmune hepatitis in the USA is reported in approximately 100,000–200,000 inhabitants.[12,14] The prevalence of autoimmune hepatitis in association with primary Sjögren's syndrome is 4–47%.[7] Autoimmune hepatitis is categorised into two types: Type 1 and Type 2 autoimmune hepatitis.[11,15] Type 1 autoimmune hepatitis is generally characterised by the detection of ANA, smooth muscle autoantibodies (SMA), and perinuclear-antineutrophil cytoplasmic antibodies (p-ANCA).[16,17] Type 2 autoimmune hepatitis is characterised by the detection of definitive antibodies such as anti-LKM Type 1; detection of anti-LKM antibody Type 3 or antibodies against liver cytosol specific Type 1 antigen are infrequently found.[17] Type 1 autoimmune hepatitis is the classic form of the disease that usually responds well to corticosteroids. Type 2 autoimmune hepatitis is typically seen in females, the paediatric, and Mediterranean population, and can present as severe disease, known as fulminant hepatitis. The prevalence of ANA in patients with Type 1 autoimmune hepatitis associated with Sjögren's syndrome according to Fayaz et al.[18] is 1.70–13.00%, while 1.49% of patients are found to have SMA. Tzioufas et al.[17] quoted the prevalence of ANA in patients with Type 1 autoimmune hepatitis associated with Sjögren's syndrome as 77.0–90.0%, while 6.5–62.0% of patients were detected with anti-smooth muscle antibody.[17,18] Gatselis et al.[11] quoted the prevalence of anti-LKM Type 1 antibody in patients with Type 2 autoimmune hepatitis associated with Sjögren's syndrome as 0–10%, while the prevalence of anti-LKM Type 3 antibody as 5–10%.[11] Zeron et al.[19] quoted 0 prevalence of autoimmune of anti-LKM Type 1 antibody type 1 as none of the patients had been detected with this autoantibody.[19]

Clinical manifestations of autoimmune hepatitis vary with the severity of disease, ranging from no visible symptoms of liver disease to severe or acute fulminant hepatitis. The foremost assessment is either normal, or symptoms of chronic liver disease present in the form of hepatomegaly, splenomegaly, palmar erythema, and spider naevi. When the disease precipitates it can lead to the development of ascites, oesophageal varices, portal gastropathy, and cytopenias.[20,21] These features were also present in this case, with portal gastropathy causing massive splenomegaly and ultimately cytopenias. Autoimmune hepatitis shows a bimodal age of outbreak with children and adolescents at one age, and middle-aged females at the fourth–sixth decade of life, especially after menopause.[22] Diagnostic criteria cited for autoimmune hepatitis is a scoring system devleoped by the International Autoimmune Hepatitis (AIH) Group and the International Association for the Study of the

Liver (IASL).[12] Laboratory investigations of Sjögren's syndrome comprise biochemical and immunological tests, including levels of liver enzymes along with plasma levels of IgM, IgG, and IgA. Immunological tests, including the detection of ANA, anti-smooth muscle antibody, and anti-LKM, are done by direct immunofluorescence assay. Percutaneous liver biopsy is also performed.[7,10] Histological findings of autoimmune hepatitis show interface hepatitis, lymphoplasmacytic infiltrate, and rosette formation of liver cells, plasma cells, and piecemeal necrosis.[2,12] Treatment options to cure autoimmune hepatitis include monotherapy or a combination of corticosteroids, such as prednisolone, along with immune-modulating agents, such as azathioprine.[12]

Complications of autoimmune hepatitis include rheumatoid arthritis, Hashimoto's thyroiditis, and Sjögren's syndrome, along with unusual complications of antiphospholipid antibody syndrome.[23] In this present case, there were vague sicca symptoms in conjunction with a moderate level of ANA and anti-Sjögren's syndrome-A antibody, a low titre anti-Sjögren's syndrome-B antibody, and an ultrasound suggestive of Sjögren's. Most patients with Sjögren's disease will have a strongly positive ANA and anti-Sjögren's syndrome-A antibody at very high titres; hence, the symptoms described may be of secondary Sjögren's disease in association with autoimmune hepatitis. There was no evidence of renal involvement with normal creatinine levels; however, systemic lupus erythematosus was in the differential diagnosis and might be the cause of secondary Sjögren's disease, but the antibody panel showed negative double-stranded-DNA.

CONCLUSION

The overall frequency of Type 2 autoimmune hepatitis is less than that of Type 1 autoimmune hepatitis, but it can be associated with autoimmune diseases such as Sjögren's syndrome. Although the majority of the association between autoimmune hepatitis and Sjögren's syndrome reported in the literature has been with Type 1 autoimmune hepatitis, this case presented with positive anti-LKM antibodies and with Sjögren's syndrome, making it a very rare and novel association with Type 2 autoimmune hepatitis.

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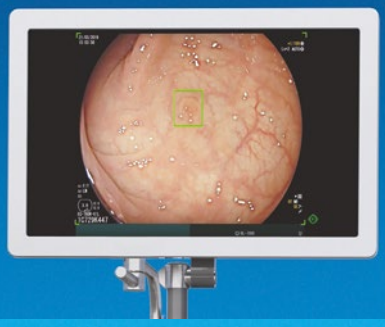

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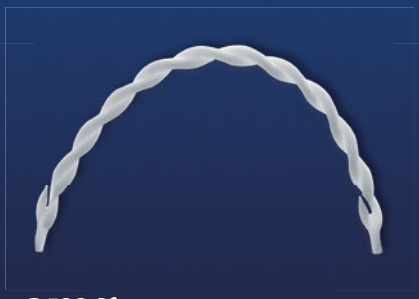

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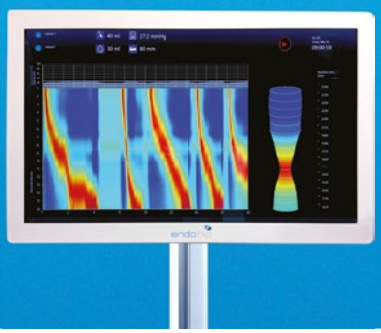

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The Benefits of Testosterone Therapy in Poor Ovarian Responders Undergoing *In Vitro* Fertilisation (IVF)

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Abstract

Introduction: Poor ovarian responders are the most challenging patients in reproductive medicine and no successful treatment has been proposed. Androgens are thought to play an important role during early folliculogenesis and diminished levels are associated with decreased ovarian sensitivity to follicle-stimulating hormone. This study aimed to determine whether pretreatment with testosterone improves the results in poor responders undergoing *in vitro* fertilisation (IVF).

Materials and methods: This observational pilot study enrolled 33 poor responders undergoing IVF. Eleven patients were pretreated with 250 mg intramuscular testosterone and compared to a control group of 22 patients. The participants were tested for free testosterone, dehydroepiandrosterone sulfate, sex hormone binding globulin, and anti-mullerian hormone (AMH).

Results: The two groups had similar baseline characteristics. Significant improvement was reached in the hormones free testosterone, dehydroepiandrosterone sulfate, and sex hormone binding globulin in the testosterone-pretreatment group. No difference was detected in antral follicle count (5.06 versus 4.24); AMH (0.51 versus 0.53), mature oocytes (2.2 versus 2.32), and the number of embryos (1.2 versus 1.33) between the study and control groups, respectively. There was a slow improvement in fertilisation rate but without any significance (62.97% versus 57.61%). However, the cancellation rate of the ovarian stimulation was much greater in the control group (18.18%) in comparison with the study group (0.0%). Pregnancy rate (PR) in the testosterone group was higher than controls (PR per cycle: 27.3% versus 4.6; p=0.09).

Conclusion: Based on the limited number of patients studied, pretreatment with testosterone seems to improve PR and cancellation rate in poor responders but failed to affect antral follicle count, AMH, and the number of mature oocytes and embryos. Given these results, further research would provide more certainty.

INTRODUCTION

The most challenging patients for fertility care providers are the poor ovarian responders (POR). During the years, many attempts have been made to define the profile of POR but the most useful classifications remain the Bologna criteria 2011 and the later Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria.[1-5] In 2016, POSEIDON[6] were developed, which are a more specific and more flexible prognosis concept for improving the management of patients undergoing assisted reproductive technologies. The proposed POSEIDON stratifies patients into four groups on the basis of age (over and under 35), anti-mullerian hormone (AMH) levels, and antral follicle count (AFC); Group 1 and 2 are defined as suboptimal responders with good prognosis and Group 3 and 4 as low responders with poor prognosis. This classification was created to serve as a guide to personalise treatment protocols and improve the management of low-prognosis patient to maximise *in vitro* fertilisation (IVF) success.

Reproductive plans are often delayed and the proportion of patients with POR has become massive. Different pharmacological approaches have been proposed to improve the outcomes of IVF treatment but none have been established with certainty. In recent decades, there has been an increased interest in the role of androgen supplementation while undergoing IVF. In 2000, Casson et al. first suggested that patients with POR could benefit from androgens.[7] Thereafter, a series of studies of patients with POR reported that dehydroepiandrosterone (DHEA) supplementation not only improves oocyte yield but also positively affects the egg and embryo quality and IVF pregnancy rate (PR).[8-17]

However, recent large-scale randomised trials have failed to confirm this, and highlight free testosterone (T) levels as a more appropriate androgen in the improvement of the ovarian reserve.[18,19] It has been established that there is a direct T effect on the ovaries through androgen receptors (AR), and sufficient AR are necessary for normal follicular development and function.[17,18]

Granulosa cells (GC) express AR at early stages of folliculogenesis, with these stages of follicle maturation occurring months before ovulation. Given this, it would be logical to assume that longer androgen supplementation would be associated with better results for follicular activation and recruitment, and thus reach a larger pool of gonadotrophin-sensitive follicles and respectively more oocytes, which appear to improve reproductive outcomes.[19,20]

Certain modalities have been tested through different studies and trials. In daily practice, the most commonly used T agents, before or during ovarian stimulation, are transdermal testosterone. Some meta-analyses evaluated the effect of different doses or duration of treatment, but the most common duration of therapy was 21 days.[19,20] The physiological cycle of the transition of primordial to preantral follicles lasts for 290 days, but in this process the levels of expressed AR are low. However in the preantral stage of follicular development, androgens, through a synergistic interaction between nuclear and extranuclear signalling, induce the expression of AR in GC, contributing to follicular survival and growth. Simultaneously, this increases follicle-stimulating hormone (FSH) receptors (FSHR) that enhance the sensitivity of preantral follicles toward FSH actions. These androgen–AR actions together promote preantral follicle growth and transition to antral stage, taking at least 65 days (Figure 1).[21,22]

Beyond the above-mentioned data, this study aimed to investigate the effects of testosterone pretreatment on the ovarian reserve and IVF outcome in patients with POR but with longer exposition and higher dosage of testosterone administered intramuscularly.

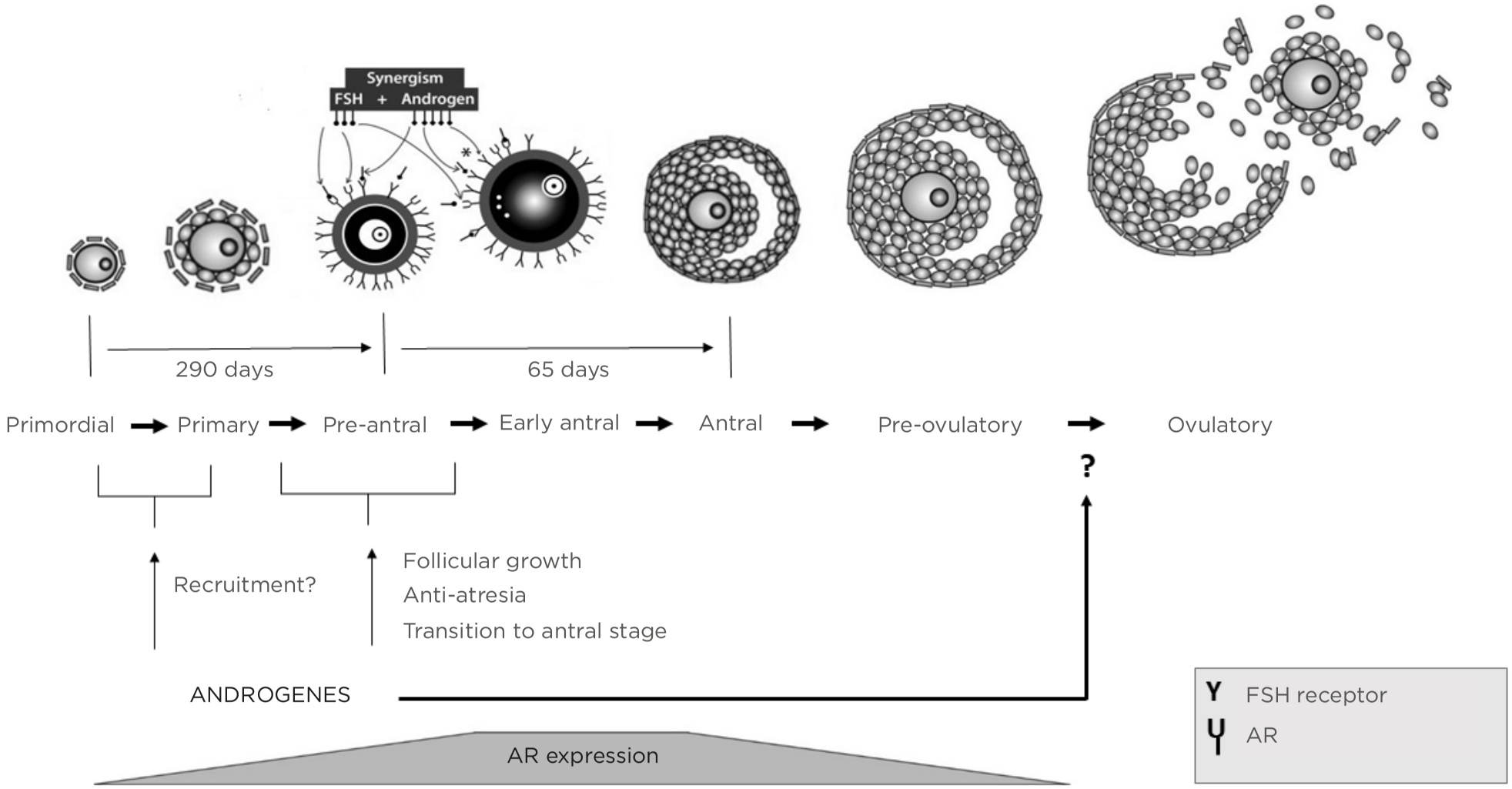

Figure 1: Folliculogenesis and physiological actions of androgens in follicular development, with synergism between androgens, AR, and FSH in preantral follicle activation and maturation.

AR: Androgen receptor; FSH: follicle-stimulating hormone; FSHR: follicle-stimulating hormone receptor.

Adapted from Gleicher N et al.[21]

MATERIALS AND METHODS

An observational pilot study was performed at the IVF department of a private obstetrics and gynaecology hospital. The inclusion criteria were female patients with POR who had at least one previous failed or cancelled IVF cycle and met criteria for POSEIDON Group 4.[5,6] The patients in the study had a mean age of 39.4 years, mean AMH levels of 0.52 ng/mL, AFC <5, number of retrieved oocytes <5, and level of oestradiol on the day of human chorionic gonadotropin therapy <1,200 pg/mL. No significant difference was observed in demographic and baseline characteristics between the two groups. Exclusion criteria were uterine malformations, hydrosalpinx, FSH >20 IU/L, or severe male factor.

Pretreatment with Testosterone in the Study Group

In all eligible patients, following signed informed consent, 250 mg of intramuscular testosterone was administered twice for 6 weeks. The active substances in the injections were testosterone propionate 30 mg, testosterone phenylpropionate 60 mg, testosterone isocaproate 60 mg, and testosterone decanoate 100 mg.

Ovarian Stimulation Protocol

IVF embryo transfer procedure was performed in all patients. A gonadotropin-releasing hormone antagonist protocol was used for the ovarian stimulation. After confirming baseline blood levels and excluding any functional ovarian cysts, ovarian stimulation with follitropin alfa was started on Day 2 of the menstrual cycle and 4 weeks after the last testosterone application. Patients in the control group underwent the same protocol, without receiving testosterone pretreatment. During ovarian stimulation, regular transvaginal ultrasound scanning and monitoring of serum concentrations of luteinising hormone, oestradiol, and progesterone were undertaken. Ovum pick-up was performed 34–36 hours after the subcutaneous administration of 5,000–6,500 IU human chorionic gonadotropin for the ovulation trigger, and the matured oocytes were fertilised by an intracytoplasmic sperm injection. All embryos were transferred at the cleavage or blastocyst stage. Luteal support was performed via transvaginal administration of progesterone, starting on the day of ovum pick-up.

Hormonal Measurements

All patients were tested for T (nmol/L), DHEA sulphate (DHEA-S) (µmol/L), sex hormone binding globulin (SHBG) (nmol/L), and AMH (ng/mL). Patients eligible for T treatment were those with SHBG <80 nmol/L, T less than one-third of the normal range, and DHEA-S within the normal range. All hormones were analysed before and after T treatment in the tested group.

Outcome Measures

The primary outcome measure was the PR. The secondary outcome measures included the number of antral follicles, developing embryos, and mature (M2) oocytes (COC); fertilisation rate (FR); and cancellation rate (CR). Additionally, changes in the serum hormonal levels of AMH, T, SHBG, and DHEA-S were observed.

FR was calculated by dividing the number of fertilised oocytes by the number of M2 oocytes. Embryo quality was assessed according to morphological criteria based on the assessment of the blastomeres and the degree of blastomere fragmentation. Clinical pregnancy was defined as the presence of an intrauterine sac with fetal heart palpitation.

Sample Size

The above-described criteria were observed for the selection of patients who were able to receive testosterone therapy. From all tested patients, 33 met eligibility criteria and, after obtaining their informed consent, 11 patients were enrolled in the T pretreatment group and 22 in the control group.

Statistical Analysis

Statistical analysis was performed. The odds ratio, with its standard error and 95% confidence interval, were calculated. For test of significance, the p value was calculated and statistical significance was considered at $p<0.05$. All analyses were performed with MedCalc® statistical software.

RESULTS

All 33 patients were divided into two groups: the study group of 11 participants received pretreatment with 250 mg intramuscular testosterone twice, every 3rd week and the other 22 participants formed the control group without pretreatment.

Table 1: The main results in the testosterone-pretreatment group and in the control group.

	T-pretreatment group	Control group	OR (95% CI)	p value
AFC	5.06	4.24		p=0.4
AMH (ng/mL)	0.51	0.53		p=0.9
COC (M2 oocytes)	2.20	2.32		p=0.9
Total number of embryos	1.20	1.33		p=0.6
Mean number of transferred embryos	1.33	1.05		p=0.5
FR (%)	62.97	57.61	1.32	p=0.6
CR (%)	**0**	**18.20**	**5.60**	**p=0.042**
PR (%)	**27.30**	**4.60**	**7.88**	**p=0.093**
SHBG (nmol/L)	57.44	104.77		p=0.02
T (nmol/L)	28.97	0.99		p<0.0001
DHEA-S (µmol/L)	10.13	5.29		p=0.0086

AFC: antral follicle count; AMH: anti-mullerian hormone; CI: confidence interval; COC: cumulus–oocyte complex; CR: cancellation rate; DHEA-S: dehydroepiandrosterone sulfate; FR: fertilisation rate; OR: odds ratio; PR: pregnancy rate; SHBG: sex hormone binding globulin; T: free testosterone.

The main demographic and baseline characteristics of all patients were very similar, including age, BMI, causes and duration of infertility, AFC, DHEA-S, T, SHBG, and AMH, with a mean BMI of 25.6 and mean age of 39.6 years. A significant improvement was reached in the levels of T, DHEA-S, and SHBG in the T pretreatment group (Table 1). No differences were detected in the AFC between the groups (5.06 versus 4.24), AMH (0.51 versus 0.53), number of M2 oocytes retrieved (2.20 versus 2.32), and the total number of embryos (1.20 versus 1.33) (Table 1). The analysis showed a slow improvement of the FR in the study group but without a significance (62.97% versus 57.61%, respectively; p=0.6). However, the CR of the ovarian stimulation in one cycle was much larger in the control group in comparison with the study group (18.8% versus 0.0%; p<0.05). The results showed an almost eight-fold increase in the odds of pregnancy after T treatment in comparison with no pretreatment; PR per cycle in the interventional group was 27.3% and 4.6% in the control group, with odds ratio 7.88 in 95% confidence interval (p=0.09) (Table 1 and Figure 2).

DISCUSSION

There is evidence that testosterone levels in individuals with diminished ovarian reserve decrease significantly, irrespective of age or premature ovarian ageing.[23,24]

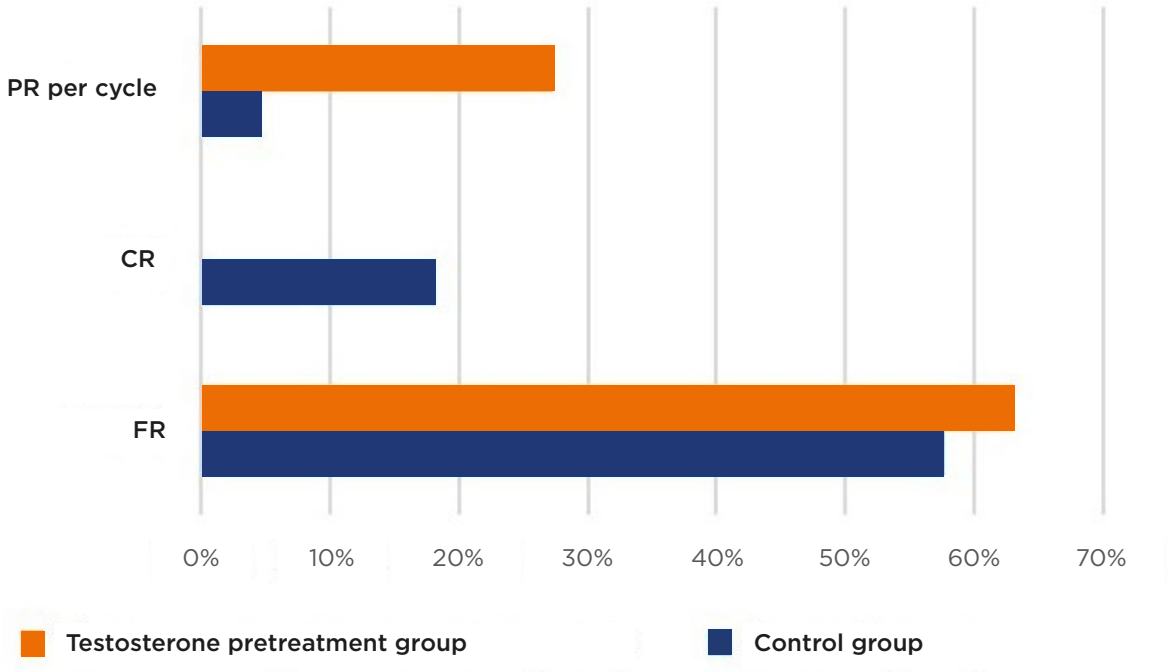

Figure 2: Pregnancy rate, cancellation rate, and fertilisation rate results.

CR: cancellation rate; FR: fertilisation rate; PR: pregnancy rate.

According to the 'Two cell, two gonadotropin' theory of ovarian steroidogenesis, oestrogen's synthesis in GC originates from androgens from thecal cells. Over the years, understanding of the effect of androgens on the ovary process and female fertility has developed significantly. Androgens are considered to be detrimental to ovarian function, and sufficient androgen actions through the AR are necessary for normal follicle development and function.[21,25] AR expression can be found in GC of mostly immature preantral and early-antral follicles; thereafter, they decrease with advancing follicle maturation, suggesting the importance of androgens mainly in the early stages of follicle maturation[21,22,26] (Figure 1). Many studies demonstrate androgens as essential for follicular recruitment, follicular growth, and reduction in GC apoptosis leading to an increase in the number of growing follicles.[27-29] Additionally, AR actions induce the expression of the micro-RNA miR-125b that decreases pro-apoptotic proteins. It has been proposed that in the ovary androgens maintain a certain level of miR-125b expression, essential for the balance between follicular survival and atresia.[30]

Furthermore, androgens have been found to induce FSHR mRNA expression during preantral to antral follicle progression, whether this induction by androgens is mediated through androgen–AR response or by direct synergism between androgens and FSH in the ovary. This suggests that androgen stimulation enhances follicular sensitivity toward FSH actions by increasing FSHR levels, which potentially contributes to follicle growth (Figure 1).[31-36]

It is known that androgens, acting via the AR, may also regulate the expression and action of key ovarian growth factors during different stages of follicle growth, which indicates that an intraovarian growth factor system plays an essential role in ovarian follicular development, regulated by androgen–AR actions.[37] A study demonstrated that during the ovulation process the androgen–AR pathway also plays a role in the last stage of folliculogenesis. Androgens produced by the luteinising hormone surge are likely to act through the AR and may be involved in the ovulatory process by directly regulating the expression of *COX2* and *AREG* genes and their actions (Figure 1).[38]

Taking into consideration the above-mentioned evidence, long pretreatment with testosterone could be beneficial for ovarian response. Despite the significantly higher testosterone level after 6 weeks of administration compared with no pretreatment, no statistically significant changes were detected in this study in the mean number of AFC (5.06 versus 4.24, respectively), mature oocytes retrieved (2.2 versus 2.32), and total number of embryos (1.2 versus 1.33) (Table 1). In agreement with this study, Massin et al.[39] performed a randomised controlled trial with 49 patients and found a nonsignificant increase with testosterone pretreatment (10 mg/day for 15–21 days) on the number of COC. Six years later, a systematic review and meta-analysis was performed by Gonzalez-Comadran et al.[40] and no differences were observed regarding the number and quality of the oocytes retrieved. In 2016, 26 patients were randomly pretreated with T, but it failed to increase the COC.[41] Contrary to this, Noventa et al.[42] observed that a higher number of total oocytes, M2 oocytes, and total embryos were developed after T therapy. The same was shown by Kim et al.,[43,44] in a significant improvement in the number of COC retrieved, and showing that the level of success is time dependent (COC retrieved with pretreatment for 2 weeks: 4.3 versus 1.6; for 3 weeks: 5.3 versus 2.0; and for 4 weeks: 5.8 versus 1.9). These inconsistent results highlight the possibility that the evaluated studies might differ by the type of substance, the timing and the duration of the treatment, and by the mechanism of action.

Apart from the idea that androgens may regulate AMH and that some studies show a possible positive relationship between androgens and AMH levels in follicular fluid, the direct evidence of androgen-induced AMH expression and its underlying mechanism in GC are still lacking.[45] The same result was observed in this study where no difference in serum AMH was observed after T therapy (0.51 versus 0.53). Vuong LN et al.[46] performed long-term intraovarian androgen priming but also did not find any significant effect on AMH level. However, despite androgens improving recruitment and activation of prenatal follicles and the fact that AMH is synthesised in GC, there are insufficient studies observing the effect of testosterone on AMH.

This study revealed a significant improvement in the CR of ovarian stimulation in the T group (0.0% versus 18.2% in the control group; $p<0.05$). This is important in impacting time, money, and patients' hope. This effect of testosterone could be explained as a result of the increased levels of FSHR mRNA in GC after androgen supplementation and supports the fact that androgens enhance follicle responsiveness to FSH, particularly in early antral stages, and the theory of a synergistic effect of androgens with FSH over folliculogenesis.[32,34]

Despite these conflicting results, the vast majority of studies claimed greater clinical PR after testosterone therapy, consistent with this study's results (PR per cycle: 27.3% in T group versus 4.6% in control group; $p=0.09$, close to significance). Noventa et al.[42] performed a meta-analysis of available randomised controlled trials on the effect of transdermal testosterone, demonstrating a higher clinical PR and live birth rate. Recently, Vishwakarma et al.[47] reported an improvement in the numbers of cryopreserved embryos per cycle and hence the cumulative PR.

Limitation and Strengths of the Study

This observational pilot study has some potential limitations that need to be considered; mainly, the relatively small number of patients included. This low rate of events decreases confidence in the results. Secondly, there are no other trials with intramuscular application of testosterone, so comparison was made with other types of administration which could give some bias. However, this limitation simultaneously could be counted as a strength of the study, because it is the first report of this type. In the literature, the most common route of administration was transdermal and only one other study reported an alternative method: intraovarian priming.[46]

CONCLUSION

Long-term testosterone pretreatment could be considered promising in IVF treatment of patients with POR. The findings of this study show improvement of the PR and CR. No effect was observed on AFC, AMH, M2 oocytes retrieved, and the total number of embryos. Due to the limitations described above, more studies should be performed with a larger population and better adjustment with ovarian physiology by dose, timing, type, and duration of the testosterone therapy.

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What Makes the Lung Unique – Tissue-Specific Immunity in the Respiratory Tract

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Abstract

The immune system constitutes a critical mechanism of the human body to preserve health and mitigate disease. In the lung, immunity is seen as a critical driver in many respiratory diseases, in particular in those characterised by aberrant inflammation, such as chronic obstructive pulmonary disease, fibrosis, and asthma. In this review, the specialised set of immune cells and lung tissue-specific regulators, including key cytokines such as granulocyte-macrophage colony-stimulating factor and transforming growth factor β, that control immune responses in the respiratory tract will be discussed. Furthermore, the current understanding of the impact of key environmental components such as the role of oxygen and lung microbiota on lung immunity will be highlighted. The goal is to identify the unique aspects of lung immune biology to facilitate insights into the aetiology of common lung inflammatory diseases and to provide the basis for a deeper mechanistic understanding of the underlying immune processes. Finally, key future avenues of research such as using more comprehensive quantitative approaches for elucidating molecular disease mechanisms as well as the potential to exploit tissue-specific regulators of immunity for therapy of lung inflammatory disorders will be discussed.

INTRODUCTION

As the mediator of gas exchange, the lung is an essential organ for mammalian survival and has thus evolved a diverse and intricate set of defence mechanisms to deal with challenges and still maintain its crucial functions. It has the second largest surface area of all human tissues and therefore has strong and constant exposure to the environment, including inhaled pathogens, allergens, particles, as well as resident micro-organisms. Similar to every organ system, the lung has a unique set of immune cells which, together with structural cells such as epithelial cells, form an interconnected network that orchestrates lung immunity. These cells include innate immune cells such as lung resident dendritic cells (DC), alveolar macrophages (AM), interstitial macrophages (reviewed in more detail here[1]), as well as basophils and mast cells. Both lung macrophages and DC together form a first line of defence against pathogen invasion while at the same time inducing some level of tolerance to prevent unnecessary inflammation. In addition, the lung also contains a high number of Type 2 innate lymphoid cells (ILC2) as well as

other innate-like lymphocytes such as γδ T cells which play important roles in mediating lung immunity in health and disease. In recent years, the immune functions of various nonhaematopoietic cells as first sensors and transmitters of immune-relevant signals have also become apparent. While many of the underlying molecular mechanisms remain unclear and are being intensely investigated, all together the respiratory immune system reacts to internal and external challenges, promoting tissue homeostasis and repair but also contributing to acute and chronic pulmonary disease. Indeed, the lung has several intrinsic features such as high levels of oxygen that have a profound impact on the configuration of its immune system and its activity in respiratory illnesses. In this review, the current knowledge of how tissue immunity in the lung is regulated by these unique features including key host and environmental factors and how this orchestrates lung immune responses in health and disease will be discussed.

KEY ENVIRONMENTAL FACTORS REGULATING IMMUNITY IN THE LUNG

Oxygen

Because the function of the lung is to mediate gas exchange, the organ represents a unique environment with very high levels of oxygen compared to other tissues. This has a direct impact on lung immunity and disease (Figure 1). Firstly, transcriptional regulators that have activity directly linked to oxygen levels, in particular the hypoxia-inducible factor (HIF) family, play a key role in regulating lung disease in different contexts.[2] HIF have been shown to be particularly important in regulating the development of pulmonary hypertension (PH) where local differences in oxygen levels can lead to activation of HIF1α which in turn promotes remodelling of the lung vasculature by inducing smooth muscle cell thickening.[2] Furthermore, HIF2α induces pathological changes in endothelial cells in this context.[3] It is now increasingly understood that immune cells play a key role in PH pathogenesis as HIF1α was found to promote disease in a myeloid cell intrinsic manner.[4] Furthermore, there is evidence that the key regulators of HIF1α activity von Hippel-Lindau protein and prolyl-4-hydroxylase domain 3 directly regulate AM development and function.[5,6] Furthermore, HIF1α activation in myeloid cells was found to promote asthma.[7,8] A potentially detrimental role of HIF1α in promoting lung inflammation is becoming apparent in sarcoidosis in which it regulates inflammatory cytokines IL-1β and IL-17,[9] and in chronic obstructive pulmonary disease (COPD), serum HIF1α is emerging as a useful biomarker for tracking disease progression.[10] Conversely, while its role in lung infection is largely unclear, HIF1α has been shown to promote survival in chronic tuberculosis infection.[11] More research is warranted in understanding its role in immunity to other pathogens such as respiratory viruses.

Apart from directly regulating some host transcription factors, high oxygen levels in the lung oxidise the surfactant lipids continuously produced by Type II alveolar epithelial cells to facilitate gas exchange. In general, oxidised phospholipids are key regulators in inflammation, but are of particular importance in the lung because of the high availability of oxygen. Several mechanisms exist to control their levels, especially uptake and degradation by macrophages via scavenger receptors.[12] Oxidised phospholipids were shown to negatively impact AM function[13] but promote tissue repair after acute lung injury via activation of key transcription factor nuclear factor erythroid 2–related factor 2.[14,15] Despite their high abundance in the lung, their role in many respiratory diseases is still completely unknown and more work is necessary to understand the importance of individual oxidised lipid species on lung inflammation.

Lung Microbiota

The lower respiratory tract harbours a unique microbiota, distinct in composition from other body sites such as the skin or the intestine.[16] Indeed, the microbial composition in chronic lung disease is profoundly changed in conditions such as pulmonary fibrosis[17] or COPD.[18] While the functional impact of the respiratory microbiota on lung immunity is still unclear, bacteria are found in close proximity to lung epithelial cells,[19] suggesting an intimate relationship. Furthermore, there is emerging evidence that the microbiome in general has a broad impact on lung immunity, including influencing susceptibility to infection and development of asthma.[20]

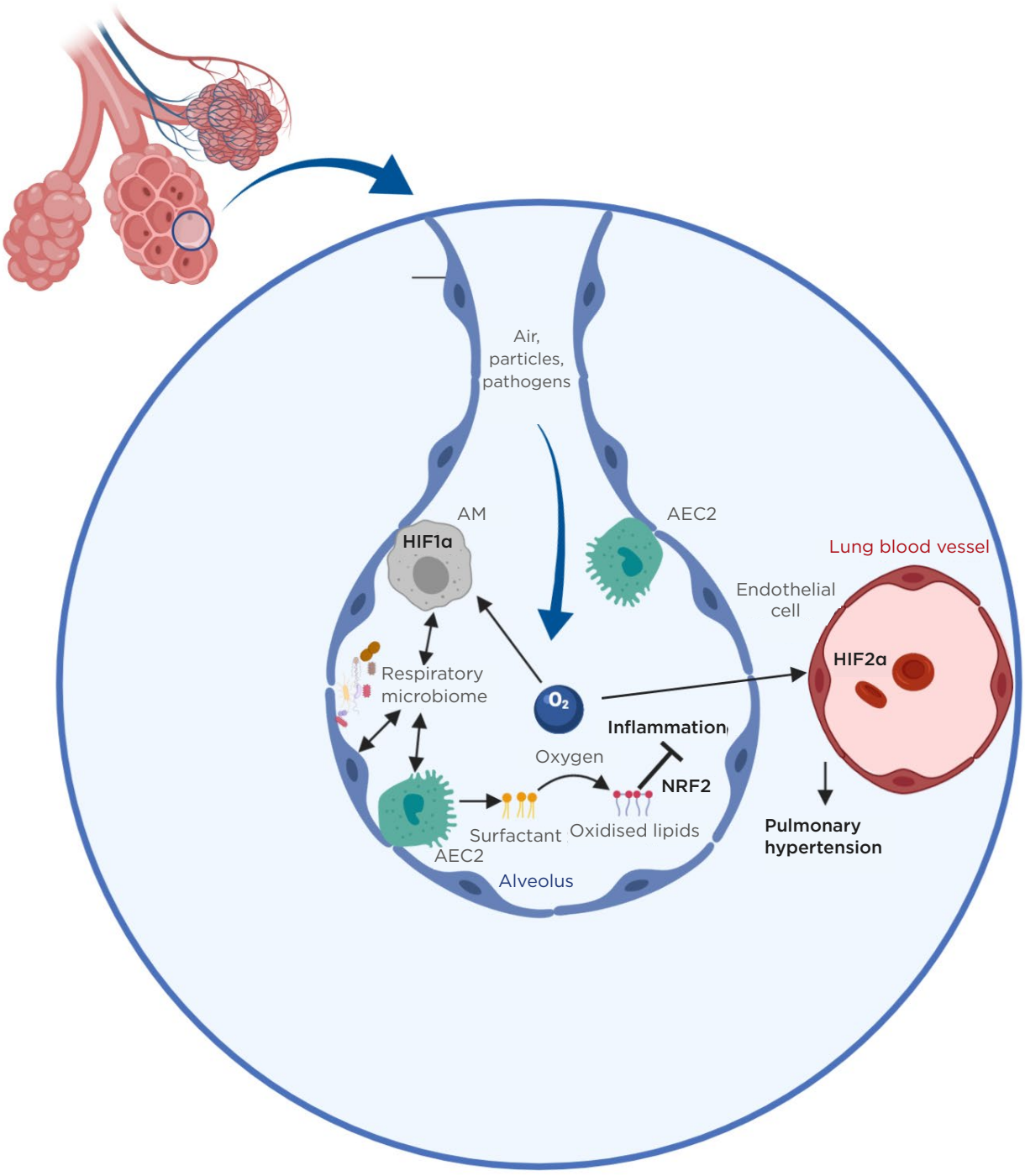

Figure 1: Environmental factors as important regulators of lung immunity.

Shown are key environmental factors controlling lung immunity. The roles of oxygen and the respiratory microbiota are depicted.

AEC2: Type II alveolar epithelial cell; AM: alveolar macrophage; HIF1/2α: hypoxia-inducible factor 1/2α; NRF2: nuclear factor erythroid 2-related factor 2.

While mechanistic insights are still largely lacking, there is some evidence that diet-induced changes in the intestinal microbiota lead to production of microbial metabolites such as short-chain fatty acids which in turn, via the circulatory system, control the development of allergic disease in the lung.[21]

Furthermore, such mediators can also control immunity to influenza virus infection by promoting antiviral T-cell responses,[22] which is critical for viral clearance and recovery from infection. While in some cases beneficial, the microbiota was also shown to exacerbate the development of chronic lung inflammation in models of COPD[23] as well as fibrosis.[24] Indeed, more evidence is emerging that, not just in experimental settings but also in humans, the microbiota directly contributes to disease development or exacerbation.[25] For example, this includes lung fibrosis for which lung bacterial burden in patients was shown to correlate with disease progression.[25] More research is warranted to investigate the underlying molecular mechanisms of microbiota–host crosstalk in the lung and how it contributes to pulmonary disease.

KEY HOST ORCHESTRATORS DETERMINING TISSUE-SPECIFIC IMMUNE RESPONSES

Several key host regulators of lung immunity have been discovered in recent years and how these factors regulate lung inflammation is displayed in Table 1.

Alveolar Surfactant

A particular key component in the alveolar microenvironment are surfactants, which are composed of a large variety of lipids such as phosphatidylcholine as well as several key proteins, notably the four surfactant proteins (SP) SP-A, SP-B, SP-C, and SP-D found in the alveolar space (Figure 2). By continuously producing Type II alveolar epithelial cells, the main purpose of the surfactant is to lower surface tension of the alveoli and thereby facilitate gas exchange. SP-B and SP-C are critical for maintaining this basic lung function. Congenital deficiency for SP-B, for example, is associated with severe respiratory distress syndrome in neonates.[26] Furthermore, for mutations in the gene encoding for SP-C, severe interstitial lung disease and in some cases a pulmonary alveolar proteinosis-like phenotype has been described.[27] However, in addition to these important roles in maintaining basic lung function, several key immunomodulatory properties have been described for SP-A and SP-D. When bacteria and other respiratory pathogens, as well as allergens and particles, enter the alveolar space, SP-D was shown to directly bind them through its collectin binding domain[28] and thereby contribute to their phagocytosis by lung-resident innate cells.[28] This is important for clearance of pathogens including respiratory syncytial virus.[29] Furthermore, SP-D can induce activation of lung DC, as well as promote inflammatory cytokine production and chemotaxis of macrophages.[28] SP-D also exhibits some anti-inflammatory properties in some contexts including inhibition of smooth muscle cell cytokine production as well as preventing mast cell degranulation.[28] SP-D can exist in both a monomeric and multimeric form and it remains unclear to what extent this contributes to its function in disease.[28]

Similar to SP-D, SP-A has also been shown to directly opsonise respiratory bacteria and viruses and thereby contribute to pathogen clearance.[30] However, it seems to have significant immunoregulatory properties because of its capacity to directly bind IFNγ and thereby suppress Type 1 immune responses.[30] Furthermore, SP-A can also suppress lung DC activation and maturation in different contexts.[30] While the role of the surfactant proteins in respiratory disease is increasingly studied, the importance of individual surfactant lipids in modulating the immune system remains largely unclear. More research will be necessary to investigate changing lipid landscapes in chronic lung diseases such as idiopathic pulmonary fibrosis and how this controls aberrant inflammation.

Transcription Factors: Peroxisome Proliferator-Activated Receptor γ

As a result of the lipid-rich environment, especially in the alveolar space, as described above, several host factors which are regulated by lipid ligands have been identified to play an important role in regulating lung immunity.

Table 1: Important host factors in lung immunity and their role in lung disease.

Key host factor	Important cell type(s)	Role(s)	Disease relevance
Surfactant protein A	AEC2	Opsonises bacteria and promotes phagocytosis Suppresses activation of DC Suppresses Type 1 mediated immunity	Respiratory infection
Surfactant protein B	AEC2	Facilitate gas exchange	ARDS in neonates
Surfactant protein C	AEC2	Facilitate gas exchange	PAP-like interstitial lung disease
Surfactant protein D	AEC2, AM	Binds particles and pathogens, promoting clearance Modulates immunity	Asthma Respiratory infection
PPARγ	AM, DC, T cells, EC	Essential for AM development Intrinsically promotes Th2 effector differentiation Lung DC Th2-priming capacity Anti-inflammatory role in EC	Asthma COPD
PI3Kγ	DC	Intrinsically controls development of lung DC network downstream of key receptor FLT3	Viral infection
GM-CSF	AEC2, AM, DC, granulocytes	Produced by AEC2 Controls AM and DC development Promotes granulocyte recruitment	PAP Asthma Viral infection
TGFβ1	AM, EC	Promotes tolerance but also contributes to aberrant inflammation Required for AM development Involved in lung fibrosis	IPF Asthma ARDS
IL-33	AEC2, ILC2, T cells, DC	Produced by AT2 Promotes allergic responses and aberrant inflammation	Asthma IPF COPD

The roles of key host factors controlling tissue-specific lung immunity in the steady-state and in inflammation are shown in the table.

AEC2: Type 2 alveolar epithelial cell; AM: alveolar macrophage; ARDS: acute respiratory distress syndrome; COPD: chronic obstructive pulmonary disease; DC: dendritic cell; EC: epithelial cell; FLT3: FMS-like tyrosine kinase 3; GM-CSF: granulocyte-macrophage colony-stimulating factor; IPF: idiopathic pulmonary fibrosis; PAP: pulmonary alveolar proteinosis; PPARγ: peroxisome-proliferator activated receptor γ; Th: T helper.

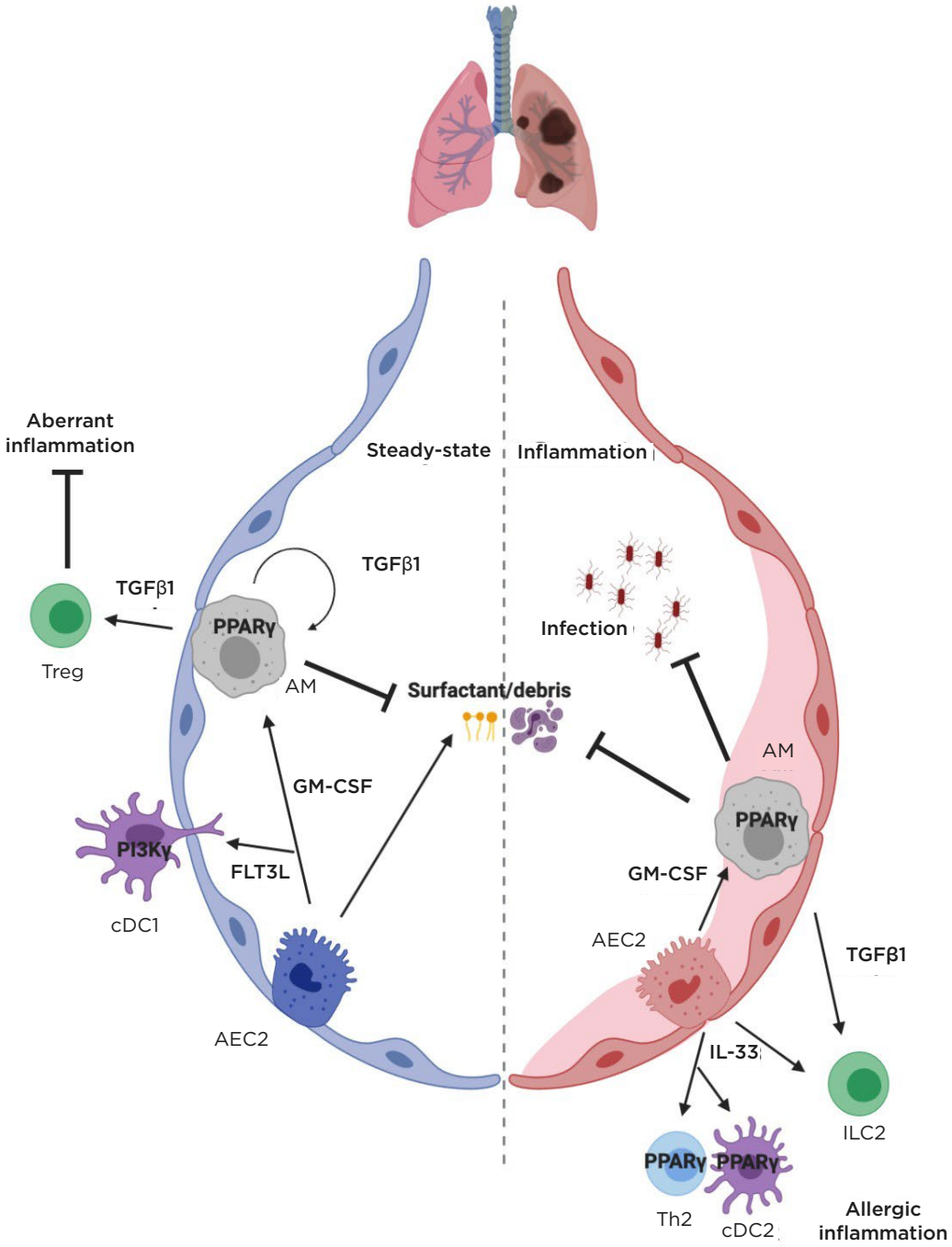

Figure 2: Key host regulators of lung-specific inflammation.

Key host factors controlling tissue-specific lung immunity in the steady-state and in inflammation are shown including transcription factor PPARγ, the cytokines IL-33, GM-CSF, and TGFβ1.

AEC2: type 2 alveolar epithelial cell; AM: alveolar macrophage; cDC1/2: conventional dendritic cell 1/2; FLT3L: FMS-like tyrosine kinase 3 ligand; GM-CSF: granulocyte-macrophage colony-stimulating factor; HIF1/2α: hypoxia-inducible factor 1/2α; ILC2: Type 2 innate lymphoid cells; PPARγ: peroxisome-proliferator activated receptor; TGFβ: transforming growth factor β; Th: T helper; Treg: regulatory T cell.

A key example in this context is peroxisome-proliferator activated receptor γ (PPARγ). This lipid-activated transcription factor is classically associated with regulating adipogenesis; however, in recent years multiple distinct functions of PPARγ have emerged in the lung.[31] PPARγ can be highly expressed by several cell types in the lung including AM,[32] DC,[33] epithelial cells,[34] as well as T cells.[33,35]

In nonhaematopoietic cells, a mechanistic understanding of PPARγ is still largely lacking but there is some evidence for a role in the development of emphysema[34] and a minor role in lipid metabolism of club cells.[36] By contrast, in AM it was shown to be crucial for their development by regulating differentiation of lung fetal monocytes after birth.[32] Perinatal induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) expression in lung epithelial cells in turn induces PPARγ in lung fetal monocytes, which then give rise to a self-renewing AM compartment.[32] Because of the essential role of AM in the maintenance of gas exchange during infection,[37] PPARγ-deficiency leads to a highly increased susceptibility to influenza virus infection despite intact antiviral immunity[37] which was associated with increased inflammation[38] as well as impaired resolution of inflammation after infection.[39] Apart from AM, PPARγ was also shown to play a key role in asthma where it is required for the induction and exacerbation of allergic lung inflammation.[33] Contrary to early indications that PPARγ agonists dampen allergic immunity,[40] PPARγ was shown to regulate the capacity of lung DC to trigger T helper (Th) 2-driven inflammatory responses, including eosinophilia and mucus production.[33] It was also found to promote IL-33-induced effector function of Th2 cells themselves,[33,35] suggesting a broader role in the development of allergic disease in the lung. Indeed, in human T cells PPARγ was shown to drive a specific Th2 effector programme characterised by high expression levels of IL-9, which in turn strongly correlates with allergic inflammation in the skin.[41] While the inducers of PPARγ expression have been increasingly identified, the lipid ligands driving transcription factor activity *in vivo* remain largely unknown and more research is warranted to define these regulators in lung immunity. However, there is increasing evidence linking PPARγ to the HIF family of transcription factors,[42] suggesting a potentially indirect regulation of PPARγ activity by oxygen, but this is yet to be addressed in a mechanistic manner.

Tissue-Specific Cytokines: GM-CSF, IL-33, and TGFβ1

GM-CSF has recently emerged as a key orchestrator of lung immunity both in homeostasis as well as in inflammation. In the steady state it is mainly expressed by Type II alveolar epithelial cells,[43] and is required for the development of AM[37,44,45] and DC[46,47] as well as to regulate the pool size of AM.[48] Indeed, congenital deficiency of GM-CSF or its receptor leads to pulmonary alveolar proteinosis as a result of the absence of AM-mediated surfactant clearance.[49] In inflammation, GM-CSF has shown to be crucial for maintenance of gas exchange during pulmonary viral infection[37] and promote survival against pulmonary bacterial pathogens.[50,51] Indeed, inhalation of GM-CSF is being explored as a potential treatment for coronavirus disease (COVID-19) in the hope of improving lung function. Furthermore, GM-CSF was shown to promote recruitment of granulocytes to the lung in allergic asthma.[47] Interestingly, the protective function of GM-CSF in bacterial infection seems to depend on the presence of the microbiota through nucleotide-binding oligomerisation domain-containing protein 2-mediated stimulation of AM.[50] This highlights the importance of key environmental factors for regulating lung immunity.

Another key element regulating the lung microenvironment is IL-33. While generally thought of as an alarmin and indicator of tissue damage, in the lung it is already highly expressed in the steady-state by lung epithelial cells and is critically important in the induction[33] and exacerbation of allergic immune responses, including the development of asthma.[52] Indeed, IL-33 single nucleotide polymorphisms are strongly associated with asthma in humans.[53] In this context, IL-33 appears to be of particular importance in early life[54] and involved in promoting virus-induced asthma exacerbations.[55] Furthermore, IL-33 is important in promoting ILC2-mediated tissue repair after infection[56] and regulatory T cell-mediated tissue homeostasis after lung injury.[57] It is also associated with disease severity in COPD[58] and promoting detrimental inflammation in lung fibrosis[59] via its effect on ILC2 and lung macrophages.[60] Overall, through its strong role in promoting lung tissue-specific Type 2 immune responses it can thus be beneficial or harmful, depending on the disease context.

Furthermore, another key cytokine controlling tissue-specific immune responses in the lung is TGFβ1; an important regulator of lung

development and in respiratory disease is classically associated with driving epithelial to mesenchymal transition in idiopathic pulmonary fibrosis.[61] Recently, however, additional important roles in regulating lung immunity in health and disease have emerged. TGFβ1 is a key driver of AM differentiation and TGFβ receptor signalling, inducing PPARγ expression together with GM-CSF which then leads to full maturation of AM.[62] AM themselves express TGFβ1 in the steady state, which is important for induction of allergic airway inflammation-suppressing regulatory T cells.[63] Conversely, TGFβ1 promotes ILC2-mediated induction of airway hyperresponsiveness[64] in asthma as well as IL-9 producing Th cells which drive lung structural remodelling in this context.[65] In pulmonary viral infection, TGFβ1 has a detrimental role attributable to its suppression of early antiviral immunity;[66] however, it also limits immunopathology later on and thus contributes to improved survival.[67] In patients with acute respiratory distress syndrome, TGFβ1 in the bronchoalveolar lavage serves as a marker predicting poorer survival, but the underlying mechanisms remain unclear.[68] Because of the complexity and the pleiotropic nature of the biology of TGFβ1, more research is necessary to understand its cell type and context-specific roles. In particular, the role of TGFβ1 in other common lung disorders, such as COPD, needs to be addressed on a more mechanistic level.

Lung Specific Signalling Factors: PI3Kγ

There is emerging evidence that various tissue cells of similar lineages use different signalling modalities to respond to key growth factors or hormones. This level of complexity in the signalling cascade downstream of common growth factor receptors is exemplified in the lung by the role of phosphoinositide receptor γ in controlling lung-specific development of DC.[69] While PI3K signalling in general is known to regulate a wide myriad of processes, there is accumulating evidence that the different types of PI3K proteins have distinct and overlapping roles in different cell types and different microenvironmental niches.[70] In the lung, DC as key innate sentinels of the immune system strictly require intact PI3Kγ to respond to the FMS-like tyrosine kinase 3 ligand,[69] a key modulator of haematopoietic immune development.[71] Deficiency in PI3Kγ leads to strongly impaired development of lung DC,[69] which translates into increased susceptibility to viral infection because of impaired induction of antiviral immunity.[72] PI3Kγ is not required for Fl3L-induced differentiation in closely related DC populations in other organs such as the skin or the intestine.[69] This suggests that lung-resident cells harbour unique signalling networks that distinguish them from cells in other tissues. While the microenvironmental factor that drives PI3Kγ expression in lung DC is still unclear, it suggests that understanding tissue-specific signalling for each given cell type is crucial and molecular mechanisms in one tissue cannot be easily translated to another organ system. This emphasises that more research is necessary to identify lung-specific signalling regulators in other key cell lung-resident immune cell types.

Lung Structure and Tissue Organisation

Another key element that determines the uniqueness of the lung immune system is the need to adapt a highly structured environment with large airways, bronchi, bronchioles, and alveoli. While the understanding of how localisation within a tissue determines immune function is still in its infancy, there is emerging evidence that in the lung the function of immune cells is dependent on their localisation. For example, antigen uptake by lung DC is primarily restricted to cells localised close to alveoli, while subsequent inflammation develops more in the vicinity of the airways as a result of preferential recruitment of additional antigen-presenting cells and T cells in the context of allergic airway inflammation.[73] Indeed, it appears that the proximity to the airways directly impacts DC behaviour *in vivo*.[74] Adding further complexity to understanding the importance of local tissue organisation are changes to the tissue architecture during inflammation, such as airway remodelling in asthma or the emergence of tertiary lymphoid organs in lung infection. Understanding how the local microenvironment impacts lung immunity in health and disease is a key area of future research.

FUTURE PERSPECTIVES

Having described what unique environmental and intrinsic features regulate the respiratory

immune system, the logical next step is to identify what the implications are for the future, from a research and therapeutic perspective. To understand the overall complexity of respiratory immunity in health and disease, more systematic approaches and methodologies need to be employed and with the advent of omics technologies this is becoming increasingly feasible. Comprehensive characterisation of healthy and disease states using single cell RNA sequencing will provide a more comprehensive picture of cellular composition, cell states, and cell–cell interaction networks. Especially emerging methods that incorporate spatial features will pave the way for a more complete understanding of how lung immunity contributes to disease. Such comprehensive analyses of different organ systems will also allow a more global view of what unique factors control respiratory immune responses as opposed to other tissues. This is of importance as it will facilitate the development of targeted therapies against pulmonary diseases. Apart from using monoclonal antibodies to inhibit host-intrinsic factors, which promote respiratory pathologies, the emergence of using other approaches that modulate lung immunity in a specific manner will be crucial for the development of better therapeutic measures. Indeed, understanding how the lung immune system interacts with other complex networks such as the respiratory microbiota will be crucial to understand the complex aetiology of some lung diseases such as asthma, COPD, or fibrosis. Many novel therapeutic approaches will include manipulating the respiratory microbiota using probiotics as well as microbiota-derived small molecule metabolites. While a mechanistic understanding of the role of the respiratory microbiota is still lacking, with emerging evidence for its importance in lung disease, it will be likely of great benefit to manipulate its composition and function to achieve a more healthy configuration and prevent disease-associated dysbiosis.

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Treatment of Interstitial Cystitis/Bladder Pain Syndrome: A Contemporary Review

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Abstract

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a debilitating condition affecting approximately 3% of the female population. IC/BPS is defined as an unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms for more than six weeks duration, in the absence of infection or other identifiable cause. This condition is known to have a profound negative impact on quality of life. There are few well-studied treatment options and no cure for this condition, which is therefore challenging to treat. The purpose of this narrative review is to summarise the contemporary literature, including the Canadian Urological Association (CUA) and American Urological Association (AUA) guidelines, on various treatment options that exist for IC/BPS, including conservative therapies, oral therapies, intravesical therapies, and more invasive surgical options. Most importantly, this review highlights the need for an individualised, multimodal approach to the treatment of IC/BPS.

INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS), is among one of the most common diagnoses that give rise to chronic pelvic pain.[1] Currently, the definition referred to by the Canadian and American Urological Associations (CUA and AUA), as well as the European Association of Urology (EAU), is one offered by the Society for Urodynamics and Female Pelvic Medicine and Urogenital Reconstruction (SUFU). They define IC/BPS as "an unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms for more than 6 weeks duration, in the absence of infection or other identifiable causes."[2] The duration of pain or discomfort required for diagnosis has varied across definitions from 4 weeks to 6 months. A shorter required duration of pain is thought to facilitate earlier treatment.[3] IC/BPS is considered a diagnosis of exclusion, confirmed after the exclusion of other urological and gynaecological conditions such as urinary tract infection, malignancy, overactive bladder, and endometriosis.

Because of the progression in its definition, the epidemiology of IC/BPS has been difficult to determine. American data suggest a prevalence of between 2.7% and 6.5% of females.[4] IC/

BPS affects both females and males, though studies have shown a 5:1 female-to-male preponderance.[5] However, it is thought that there is a dramatic under-reporting of this condition in men attributable to the significant symptom overlap of chronic prostatitis/chronic pelvic pain syndrome.[6,7]

The aetiology of IC/BPS is poorly understood. Multiple theories exist, including disruption in the permeability of the urothelium lining the bladder (glycosaminoglycan deficiency), infection, autoimmune activation, mast cell infiltration, and neurogenic mechanisms. Approximately 5–10% of patients presenting with this symptom complex will be found to have ulcerations in the bladder known as Hunner's lesions (HL).[8] HL are associated with more severe symptoms and decreased bladder capacity, although it is not possible to identify patients with HL based on symptoms alone. Recommendations on the utility of cystoscopy vary between guidelines.[3] The CUA guidelines list cystoscopy as 'recommended' for all patients with IC/BPS, while the AUA guidelines consider cystoscopy 'optional'.[1] The aetiology of HL is yet to be determined. Biopsy of these lesions is mandatory to rule out other underlying disorders such as carcinoma *in situ*, urothelial carcinoma, other malignancy, nephrogenic adenoma, or eosinophilic cystitis.

Treatment must be focussed on maximising quality of life (QoL), as there is no treatment that will change the natural history of this condition or cure IC/BPS. Traditionally, the treatment of IC/BPS has been approached in an algorithmic fashion; however, there has been a shift towards treating patients based on symptom phenotypes.[1,6] A classification system, UPOINT, has been proposed as a way to direct multimodal therapy with an individualised approach. UPOINT domains include urinary, psychological, organ-specific, infection, neurologic, and muscle tenderness.[9]

In addition to symptom phenotype, treatment options should be based on the degree of QoL impairment, patient preference, availability or access, and adverse event profile. Figure 1 is adapted from the CUA guidelines on IC/BPS and offers a summary for the management options for IC/BPS.[6]

IC/BPS, whether nonulcerative or ulcerative, remains a poorly understood entity which lacks effective, evidence-based treatments. IC/BPS presents healthcare professionals with a dilemma when selecting appropriate treatment options for patients; multiple treatments already exist but the evidence supporting these treatments is often conflicting. In addition, new treatments covering a variety of therapeutic mechanisms are continuously being investigated. Decisions regarding treatment selection, and keeping up-to-date on new treatments being explored, remain a challenge for healthcare professionals. The rationale for this narrative review is to offer clinicians a practical approach to the treatment of both nonulcerative (NUIC) and ulcerative IC/BPS (UIC) and secondly, to provide a brief update on potential novel therapies for the treatment of IC/BPS.

METHODS

A literature search was carried out for this narrative review. PubMed was searched using the terms 'interstitial cystitis' and 'bladder pain syndrome' over the last 5 years. The authors searched using the filters "English", "core clinical journals", "age 19+" and "humans". In addition, recent guidelines from major urological associations, as well as other review articles, were examined to obtain pertinent references on standard recommended treatment options for IC/BPS and novel therapies.

NONULCERATIVE DISEASE

Conservative Therapies

Therapies recommended to all patients with IC/BPS, regardless of subtype, include education, dietary modifications, bladder training, and adaption of stress-management techniques. There is substantial evidence to suggest that 40–50% of patients will improve with adherence to these principles alone.[10,11] Up to 90% of patients report dietary triggers for their IC/BPS symptoms.[12] Common dietary triggers include acidic foods (i.e., tomatoes, citrus), caffeine, alcohol, spicy foods, and artificial sweeteners. Bladder training, including urge-suppression and distraction techniques, may be beneficial.[13]

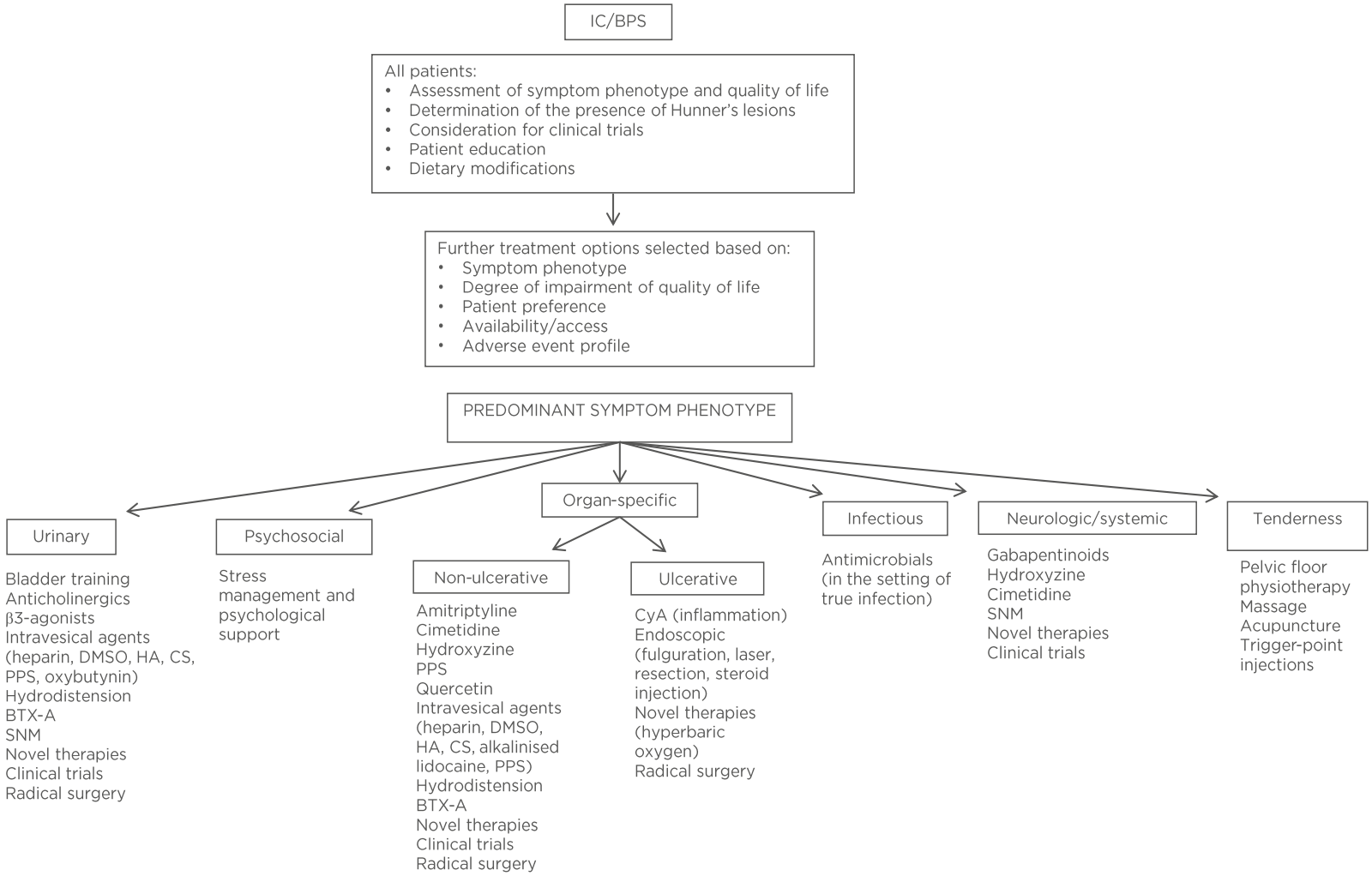

Figure 1: Proposed management paradigm for the treatment of interstitial cystitis/bladder pain syndrome.

BTX-A: OnabotulinumtoxinA; CS: chondroitin sulfate; CyA: cyclosporine A; DMSO: dimethyl sulfoxide; HA: hyaluronic acid; IC/BPS: interstitial cystitis/bladder pain syndrome; PPS: pentosan polysulfate; SNM: sacral neuromodulation.

Adapted, with permission, from the Canadian Urological Association (CUA) Guidelines.[6]

Psychological stress is known to be a trigger for IC/BPS symptoms and techniques including yoga, reduced work hours, and exercise are thought to be beneficial.[14]

Up to 87% of patients with IC/BPS will have concomitant pelvic floor muscle dysfunction and muscle tenderness.[15] These patients may also report issues with dyspareunia and bowel dysfunction. These patients have a high chance of symptom improvement and a 21% chance of cure with pelvic floor physiotherapy.[16,17] Massage techniques and physiotherapy are recommended by the CUA for these patients. These techniques may be costly and difficult to access for some patients.

Medical Management

There are a variety of oral therapies that have been studied for the treatment of IC/BPS, including amitriptyline, pentosan polysulfate (PPS), hydroxyzine, cimetidine, gabapentinoids, and cyclosporine A (CyA). In general, medications are reserved for patients who have failed a trial of conservative therapies alone.

PPS (Elmron®) is the only oral medication approved by the U.S. Food and Drug Administration (FDA) and Health Canada for the treatment of IC/BPS. PPS is an oral heparinoid and is thought to exert its effect through replacement of the urothelial glycosaminoglycan layer.[18] Multiple small, placebo-controlled, randomised controlled trials (RCT) have been completed, reporting conflicting results, possibly because of the study design. A meta-analysis, including data on >400 patients, summarised these results comparing PPS to placebo; the meta-analysis concluded a significant improvement in symptoms of pain (37%), urgency (28%), and frequency (54%), but not nocturia.[19] There is an ongoing observational trial assessing the outcomes of PPS in combination with hydrodistension.[20] The therapeutic effects of PPS may not be seen for up to 6 months. PPS may also be administered intravesically.[21] PPS is costly and may result in side effects, including diarrhoea, nausea, headache, abdominal pain, and reversible alopecia. In addition, a recent association between long-term PPS use (median 19.2 years) and vision-threatening maculopathy has been reported.[22] Given the significant improvements in up to 44% of patients and the low rate of serious adverse events, PPS has been considered an option in the CUA and AUA guidelines.[1,6]

The tricyclic antidepressant amitriptyline has anticholinergic, antihistamine, analgesic, and sedative properties. There is evidence to support the use of amitriptyline, as placebo-controlled RCT have found a statistically significant improvement in symptom scores ($p=0.005$), and urinary urgency and pain ($p<0.001$) in comparison to placebo.[23,24] Trials fail to show a benefit in doses <50 mg and side effects at this dose are common.[10] Side effects include sedation, dry mouth, and constipation. Amitriptyline is considered an option by the CUA and AUA, after conservative strategies alone have failed.

Excess production of mast cells within the detrusor muscle of the bladder, leading to histamine release, is one of the proposed causes of IC/BPS. Following this theory, the use of cimetidine, an H2-histamine antagonist, has been investigated for the treatment of IC/BPS. Its use is supported by evidence from two small, observational trials and a small RCT, with no reported side effects.[25] Symptoms of nocturia and suprapubic pain were among those most improved. Current regimes practised are 400–800 mg/day divided between two or three doses.

Hydroxyzine, another antihistamine, has been studied in a few small RCT, with conflicting results. One observational study found a 40% reduction in symptoms. In another RCT, there was minimal benefit of hydroxyzine versus placebo; however, there was a benefit with adding hydroxyzine to PPS, increasing the response rate to 40% versus 28% with PPS alone.[26] Side effects of hydroxyzine are common and may include drowsiness, constipation, dry mouth, and gastrointestinal symptoms.

Many other oral agents have been studied for treatment of IC/BPS, with very limited or conflicting results. Not all of these medications have been included in guideline statements but may be of value when treating this patient population. Patients should be informed of the lack of large trials to support the use of these medications for IC/BPS and be made aware of potential side effects. These agents include gabapentin, pregabalin, quercetin, montelukast, sildenafil, and L-arginine.[27]

Intravesical Therapy

Several agents have been studied for intravesical use in the treatment of IC/BPS. Examples of these include heparin, dimethyl sulfoxide (DMSO), multi-agent combinations, PPS, hyaluronic acid, chondroitin sulfate, lidocaine, *bacillus Calmette–Guérin* (BCG), and resiniferatoxin. There is no consensus on the frequency, duration, or dose of intravesical therapies for IC/BPS. Common side effects of intravesical therapies include mild discomfort, haematuria, and urinary tract infection. Based on evidence showing no convincing improvement in symptoms and a high side effect profile, BCG and resiniferatoxin should not be used.

DMSO is the only FDA- and Health Canada-approved intravesical agent. Its mechanism of action is thought to include anti-inflammatory and muscle relaxant effects. Data on the effectiveness of DMSO seem to be dated and conflicting. A 2007 Cochrane review reported no significant improvement over placebo;[28] however, further studies suggested there may be a role for DMSO, particularly in patients with ulcerative disease.[29] Treatment with DMSO may cause halitosis ('garlic breath' odour) or temporary flare of symptoms after the initial treatment.

Heparin is thought to exert anti-inflammatory and angiogenesis-promoting effects on the bladder mucosa. It may be used alone (20,000–40,000 units diluted in 10–50 mL of normal saline) or in conjunction with lidocaine, sodium bicarbonate, and DMSO.[6] Several studies have suggested a symptomatic improvement with heparin at a variety of doses (56–73% of patients with symptomatic improvement at 3 months).[30,31] Heparin instillations may be administered by the patient at home on an as-needed basis and represent an option for the treatment of symptomatic flares. Further research to confirm the benefit of the abovementioned intravesical therapies would be beneficial but novel, large, well-designed trials are lacking. Despite this, these therapies are used commonly. Intravesical therapies remain second-line therapies based on the AUA guidelines and are recommended in select patients based on the CUA guidelines.

Hydrodistension (Bladder Dilation)

Despite a lack of standardised technique, hydrodistension has been used for almost 100 years and is one of the most commonly used treatments for IC/BPS.[32] This technique involves performing a cystoscopic examination under general anaesthetic and filling the bladder with sterile water to its maximum anaesthetic capacity at a pressure of 80–100 cm H_2O. Theoretically, it is beneficial due to a temporary ischaemia to nerve endings resulting in a decrease in bladder pain and increased bladder capacity. There is a lack of randomised data for this technique.[33] Dated observational studies report variable findings, with a response rate ranging from 30–54% at 1 month to 0–37% at 6 months following treatment.[6] The treatment effects are not permanent and the procedure may need to be repeated. Data regarding the long-term effects of repeat hydrodistension are lacking. A contemporary systematic review evaluated the evidence for the use of hydrodistension for BPS, focussing on patient-related outcomes. Seventeen studies were included, none of which used a validated outcome measure to assess the effect of hydrodistension alone.[33] The AUA guidelines suggest hydrodistension be used as a third-line therapy and it is considered optional in select patients by the CUA.

OnabotulinumtoxinA (BTX-A)

OnabotulinumtoxinA (BTX-A) has been studied for the treatment of IC/BPS based on the antinociceptive and motor-paralytic actions of this agent. It is approved for the treatment of overactive bladder and urgency incontinence and is widely used. Multiple small RCT have been conducted on patients with IC/BPS with conflicting results. A contemporary meta-analysis of 12 RCT, including 459 patients, found a significant improvement in Interstitial Cystitis Symptom Index (ICSI) and Problem Index (ICPI) scores, pain scores, and daytime frequency in patients with IC/BPS treated with BTX-A.[34] A recent network meta-analysis of intravesical therapies for IC/BPS found that BTX-A, in comparison to instilled intravesical therapies, resulted in the greatest improvement based on global response assessment (GRA).[35] Larger RCT are required to confirm these results. Several variations to the delivery of intravesical BTX-A have been studied, including injecting BTX-A into the bladder trigone as opposed to the posterior bladder wall, and instillation of BTX-A into the bladder in a liposomal formulation.[36,37] Patients must be aware of the potential risk of

urinary tract infection, haematuria, and need for temporary clean intermittent catheterisation following BTX-A. BTX-A is also an option for patients with UIC. BTX-A is considered a fourth-line therapy for IC/BPS based on the AUA guidelines and an optional treatment based on CUA guidelines.

Sacral Neuromodulation

Sacral neuromodulation (SNM) is not approved for the treatment of IC/BPS but is used for urgency incontinence and frequency–urgency syndrome, both of which commonly occur with IC/BPS. SNM involves the implantation of a permanent tined lead into the third sacral foramina to regulate the afferent sacral nerve and modify bladder function. Multiple observational trials demonstrate a 42–95% improvement in symptoms.[38,39] Peters et al.[38] demonstrated a decrease in narcotic use following SNM implant. Long-term success rates approach 72% up to 62 months.[40] To date, RCT are lacking. Patients must be considered appropriate surgical candidates and be aware of the risks, including failure, need for surgical revisions, pain, and infection. The surgical revision rate for reasons other than routine battery change ranges from 27–50%. The AUA considers SNM to be a fourth-line therapy, while the CUA considers SNM optional in select patients. This technology may not be widely available at all centres.

ULCERATIVE DISEASE

UIC is characterised by the presence of ulcer-like lesions in the bladder lining. HL are identified on cystoscopic evaluation. The prevalence of HL ranges from 5–10%.[41,42] There is some evidence to suggest that the pathophysiology of pain associated with UIC differs from that of NUIC.[41] In addition to the therapies for NUIC, treatment with immunomodulating agents such as cyclosporine A, fulguration of ulcers, and major surgery in the form of urinary diversion are additional options for the treatment of UIC.

Cyclosporine A

CyA is an immunosuppressive agent involved in the regulation of T cells. As autoimmune dysfunction has been suggested as a potential cause of UIC, CyA has been studied for the treatment of IC/BPS at varying doses. RCT have compared CyA in addition to PPS versus PPS alone and found a superior effect with the addition of CyA (59% versus 13%; p<0.001).[43] Patients with HL appear to derive more benefit from CyA than those without ulcers (68% versus 30% response rate, respectively).[44] More recently, a systematic review assessed the treatment effect of CyA in patients with IC/BPS. Eight studies were included, three of which were RCT. The authors concluded that treatment with CyA could potentially result in long-term benefit; however, further evidence is required to confirm these findings.[45] CyA may be dosed at 2 mg/kg divided into twice daily (bid) dosing and drug levels should be monitored. Side effects are common, and patients must be monitored for renal impairment, hepatic impairment, electrolyte abnormalities, hypertension, and infection. Frequent side effects and the need for strict monitoring while on therapy has limited the use of CyA in clinical practice. However, Crescenze et al.[46] reported novel data on a large cohort of patients with UIC, of which 47% (26/55) were treated with CyA with favourable results. The AUA guidelines consider CyA a fifth-line therapy, while the CUA guidelines list CyA as an option in patients refractory to other therapies. CyA may be an appropriate therapy at centres with prior experience and supports in place to facilitate patient monitoring.

Ulcer Fulguration

Primary endoscopic ablation of HL has been used since 1971.[47] Treatment of HL has been shown to significantly decrease urinary symptoms including daytime frequency, urgency, and nocturia.[48] In a large observational study, 90% of 103 patients reported symptomatic relief, which lasted for up to 3 years in 40%.[49] Electrocautery fulguration, laser ablation, or resection of HL are accepted and recommended treatment options.[1,46] When comparing transurethral resection of HL to transurethral coagulation, Ko et al.[50] found no significant difference in HL recurrence-free time (12.2 versus 11.5 months for transurethral resection versus coagulation; p=0.735). There was also no difference in symptomatic improvement between groups, but the transurethral resection group had an increased rate of bladder injury compared to transurethral coagulation (7.9% versus 3.4%).[50] Although initial treatment of lesions often results in symptomatic relief, ulcers

recur and require retreatment in the majority of patients.

Intralesion Triamcinolone Injection

Triamcinolone is a long-acting synthetic steroid. Central injection of triamcinolone into HL at a depth of 2–3 mm has shown therapeutic benefit and is a therapeutic option for UIC.[51,52] Observational studies report a 70–91% response rate that lasted between 7 and 12 months.[46,52] Repeat injections are likely to be required and thought to be safe. Dosing consists of 1 mL vial of triamcinolone (40 mg/mL) diluted in 9 cm^3 of injectable normal saline, which can then be injected in 1 cm^3 aliquots.[7] This outpatient procedure is done through a cystoscope under general or spinal anaesthetic.

Invasive Surgical Procedures

Radical surgery in the form of urinary diversion is considered as a last resort for severe UIC. Urinary diversion may be performed with or without a concomitant cystectomy and is usually in the form of an ileal conduit. Historically, augmentation cystoplasty was performed. There are several case reports evaluating patient outcomes following urinary diversion. Andersen et al.[53] reported a 74% pain-free rate and 68% satisfaction rate following surgery. Of those who do not have a cystectomy performed at the original surgery, 17–22% will go on to require a cystectomy after urinary diversion because of ongoing pain.[53,54] Patients with identifiable disease (i.e., HL) in the bladder and those with diminished maximum anaesthetic bladder capacity based on findings at hydrodistension are more likely to find an improvement in pain and urinary symptoms following urinary diversion. The complication rate is high with this type of surgery and ranges in severity. According to the AUA and CUA guidelines, radical surgery should be considered an absolute last resort for patients with IC/BPS and reserved for patients with severe UIC.

NOVEL TREATMENTS

Novel treatments are constantly being investigated for the treatment of IC/BPS. Examples include oral therapies, hyperbaric oxygen, extracorporeal shockwave lithotripsy, stem cell therapy, and cannabinoids. Several oral therapies are being investigated for future use in the treatment of IC/BPS. Neurotropic growth factors, such as nerve growth factor (NGF), are upregulated in inflammatory conditions including IC/BPS.[55] Monoclonal antibodies directed against NGF have been under investigation including tanezumab and fulranumab; these studies have been terminated because of the adverse side-effect profile. To avoid systemic effects of this medication class, the use of a liposomal NGF delivery system is being investigated.[55]

Rosiptor (AQX-1125) is an oral SHIP-1 activator that is thought to negatively regulate the PI3K pathway to reduce an immunological reaction, acting as an anti-inflammatory. Tipelukast (MN-001) is an oral agent that inhibits phosphodiesterases and acts as a leukotriene receptor antagonist, also exerting an anti-inflammatory effect. Suplatast tosilate (IPD-1151 T) is another novel agent under investigation, based on its immunomodulating effects suppressing IgE. These, and several other agents, represent potential future treatments for IC/BPS. Recently, however, Nickel et al.[56] reported negative results from a 12-week randomised, double-blind, placebo-controlled, Phase 3 clinical trial showing no significant difference in daily bladder pain in patients treated with AQX-1125 compared to placebo. This study also highlighted the challenges of designing clinical trials for future intervention strategies for IC/BPS.

The use of hyperbaric oxygen chambers has been suggested to improve IC/BPS pain intensity by nearly 30%, with a 15% increase in single-void volumes.[57] There is some evidence to suggest that hyperbaric oxygen may be more effective for UIC compared to NUIC.[58] The effect appears to be related to enhanced O_2 delivery to the bladder mucosa during the filling phase, the phase when the majority of patients experience symptoms.

Very recently, a prospective, multicentre, double-blind RCT reported a decrease in pelvic pain scores following pelvic extracorporeal shockwave lithotripsy (N=24; 2,000 shocks; 3 Hz; maximum total energy flow density: 0.25 mL/mm^2) versus a placebo treatment (N=25; shockwave setting without energy transmission); however, the study did not meet the primary endpoint of change

in O'Leary-Sant symptom scores. No significant adverse events were detected.[59]

Based on a similar mode of action as CyA, intravesical tacrolimus has been investigated as a treatment for IC/BPS. In a recent pilot study, Mishra et al.[60] found that 54% of patients (13/24) treated with intravesical tacrolimus (0.1 mg/kg) dissolved in DMSO/sterile water reported a significant improvement in symptoms based on a global response assessment (GRA). Minimal side effects were noted and it was well tolerated.

The use of stem cells for IC/BPS has not been investigated in human subjects to date. However, stem cells have proven to be a viable option in animal models of IC/BPS.[61] Future study is directed towards introducing stem cells into trials on human subjects for various urological conditions.

Cannabinoid (CB) receptors are being investigated because of their potential for anti-inflammatory and immunomodulating effects.[62] Rodent models, followed by human studies, have confirmed that both CB1 and CB2 receptors are present in the urinary bladder.[63] Initial rodent studies demonstrated that the activation of CB1 and/or CB2 receptors reduced bladder inflammation by blocking the peripheral mechanical sensitivity that accompanies inflammatory cystitis.[64] Recently, a survey of men with chronic prostatitis/chronic pelvic pain syndrome revealed a cannabis use rate of nearly 50%. Of these, over half stated that cannabis was "somewhat/very effective" in managing their symptoms.[65] The role of CB receptors in the urinary bladder remains an area of active research.[66,67] To date, however, there is a paucity of clinical research investigating the use of cannabinoids in patients with IC/BPS.

CONCLUSION

IC/BPS represents a debilitating syndrome, consisting of pain perceived to be related to the bladder and associated with lower urinary tract symptoms. It has been shown to significantly impair QoL and the aetiology is poorly understood. There are numerous treatment options available but none that cure or alter the natural history of the disease. Treatments should be selected on an individualised basis, based on the primary symptomatology, patient preference, availability, and adverse side-effect profile. Differentiating NUIC from UIC is imperative as treatment of ulcers may result in a significant improvement in symptoms. A proportion of patients will require a multimodal approach to therapy and ongoing, supportive care.

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Putting into Perspective the Future of Cancer Vaccines: Targeted Immunotherapy

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Abstract

Pre-clinical models and human clinical trials have confirmed the ability of cancer vaccines to induce immune responses that are tumour-specific and, in some cases, associated with clinical response. However, cancer vaccines as a targeted immunotherapy strategy have not yet come of age. So, why the discordance after so much research has been invested in cancer vaccines? There are several reasons for this that include: limited tumour immunogenicity (limited targeted antigen expression, antigen tolerance); antigenic heterogeneity in tumours; heterogeneity of individual immune responses; multiple mechanisms associated with suppressed functional activity of immune effector cells, the underlying rationale for the use of immune checkpoint inhibitors; and immune system exhaustion. The success of checkpoint therapy has refocussed investigations into defining relationships between tumours and host immune systems, appreciating the mechanisms by which tumour cells escape immune surveillance and reinforcing recognition of the potential of vaccines in the treatment and prevention of cancer. Recent developments in cancer immunotherapies, together with associated technologies, for instance, the unparalleled achievements by immune checkpoint inhibitors and neo-antigen identification tools, may foster potential improvements in cancer vaccines for the treatment of malignancies.

INTRODUCTION

Cancer is a genetic and epigenetic disease of multicellularity, driving transformed cells to uncontrolled growth, invasion, and metastasis. In cancer, intracellular mechanisms controlling cellular proliferation are damaged first, which allows certain cells to progress to malignant transformation.[1] Activation of oncogenes by various genetic alterations occurs later in the transformation process when genetic instability has reached a critical level.[2] Understanding these molecular changes, along with their protein expression correlates, adds more precision to the anatomical classification of cancers and has ushered in the era of targeted therapy. This effort has led to the discovery of new targets and drugs, and the definition of new biomarkers.

The recent, dramatic success of immunotherapy for treatment of some of the most highly standard chemotherapy-resistant cancers (e.g., melanoma, lung cancer) has refocussed research on the role of the immune system as the main extracellular mechanism for cancer control. Using established databases, this new direction of research allows for the definition of different immune profiles across cancer types.[3,4] Novel biomarkers, identified using simple immunohistochemistry (Immunoscore®) or multi-omic methods, have been defined and validated, as well as led to better prognostication that may surpass the traditional tumour, node, metastasis (TNM) staging system.[3,5] A major focus of ongoing research is to determine why immunotherapies work or fail, and how they can be improved to reach their hoped-for potential as a broadly transformative treatment for cancer. Based on the presence of lymphocyte infiltrates and their types, three major immune phenotypes have emerged that correlate with response to immunotherapy: hot or inflamed, which respond well to immune checkpoint inhibitors (ICI); cold or 'immune desert,' which do not respond to ICI; and two subtypes within the altered (both excluded and immunosuppressed) immune phenotype. The immunosuppressed phenotype is also expected to respond to ICI as it has pre-existing activated immune cells in the tumour.[6,7]

It is these phenotypes that cancer-focussed vaccines aim to address. 'Hot' tumours often have a high mutational load and therefore are expected to express neo-antigens to provoke a strong immune response. Cold tumours, by contrast, are cancers that, for various reasons, have not been recognised or provoked a strong response by the immune system. Herein lies one of the limitations of immunotherapy. Characteristically hot tumours are limited and include bladder cancer, head and neck cancers, kidney cancer, liver cancer, melanoma, and non-small cell lung cancer, as well as tumours of different types with high microsatellite instability. The challenge is in the application of immunotherapy to cancers that are immunologically cold, such as glioblastomas, ovarian, prostate, and pancreatic cancer.[8] One of the most important questions for the future of immunotherapy is to determine how to make cold tumours immunoresponsive.

STRATEGIES AND APPROACHES IN CANCER VACCINE DEVELOPMENT

Interest in targeted cancer immunotherapy by vaccination has been reinvigorated by the U.S. Food and Drug Administration (FDA) approval of ICI, which has had an impact on vaccine strategies for use in cancer therapy.[9,10] Cancer vaccines, as a targeted immunotherapy strategy used for prevention (primary or secondary) or for treatment, have shown promise in preclinical animal studies, with the future aim of translation to the clinic. Some success is evident in the FDA approval of PROVENGE® (sipuleucel-T), a herpes simplex virus Type 1-derived oncolytic (T-VEC) immunotherapy that is injected directly into melanoma lesions; and in Gardasil®, which targets human papillomavirus known to cause cervical cancer. General research consensus is that a vaccine for cancer as a single entity is not practical because cancer reflects a myriad of different conditions.

The role of immunity in eradicating cancer is now considered in terms of stepwise events or the 'immunity cycle', a framework proposed by Chen and Mellman.[6,11] This cycle identifies six steps preceding the killing of cancer cells by the immune system (Figure 1). Once the immune system is activated it is expected, based on clinical and preclinical studies, that immunosuppression would ensue to stop the immune attack against the tumour.[7] Therefore, testing is underway of multiple strategies and approaches to activate the immune system for both hot and cold tumours (Table 1).

Peptide-based vaccines rely on the development of molecular tools for improving, as well as studying, peptide-based vaccines.[12] Whole-cell lysate vaccines are applicable to all patients, regardless of human leukocyte antigen (HLA) type.[13] Recombinant DNA or viral vector-based vaccines focus on design, delivery, and combination strategies that break tolerance and generate a strong immune response.[14] Dendritic cells are the most effective antigen-presenting cells for inducing T-cell proliferation, activation, and cross priming.[15] Tumour-associated carbohydrate mimetics are peptides that mimic the carbohydrate three-dimensional configuration on certain cancer-related proteins.[16,17]

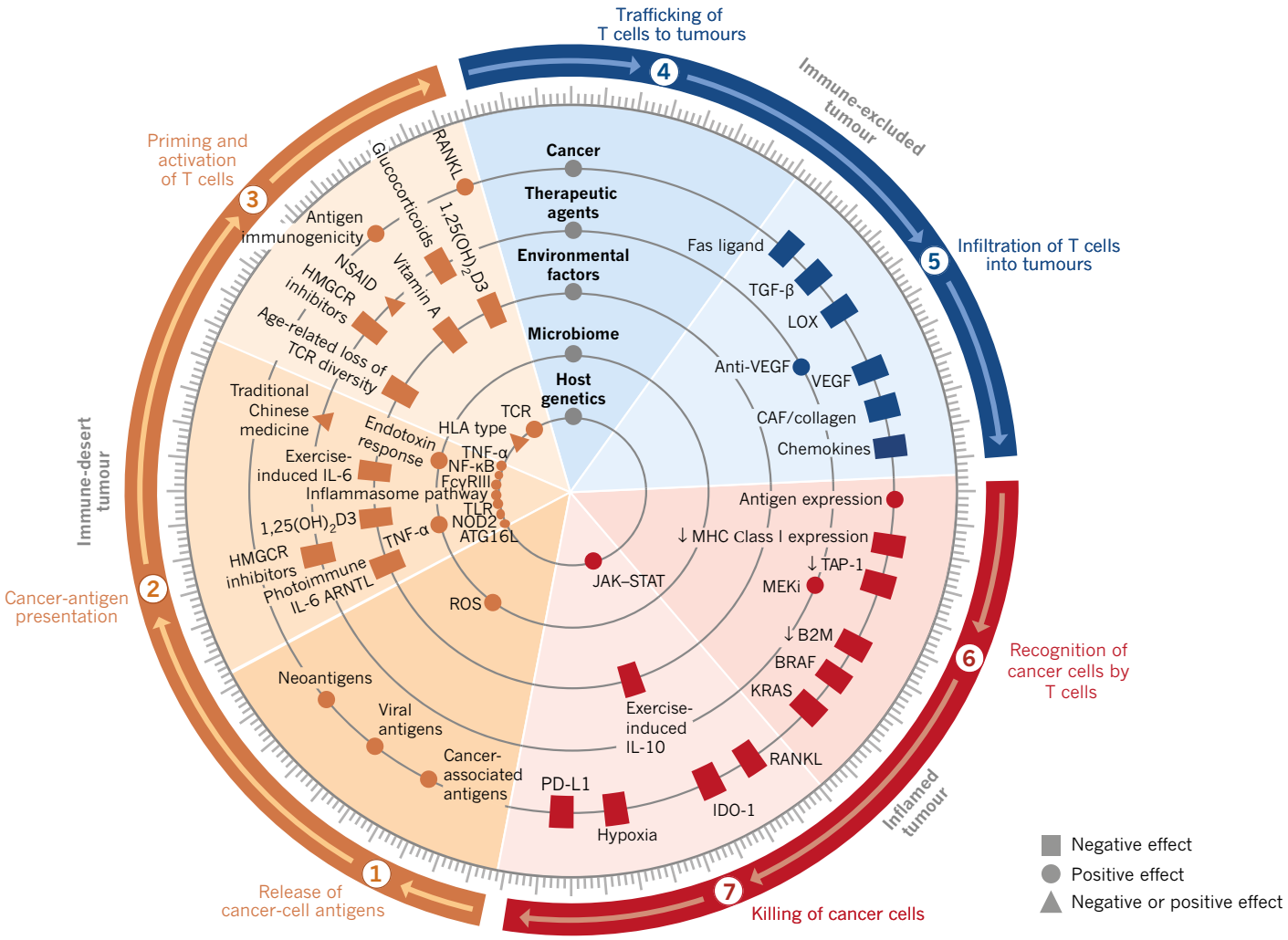

Figure 1: Multiple factors (tumour, host, and environment) affect each step of the immunity cycle.

The immune cycle can be correlated with the different immune phenotypes, and molecular and cellular abnormalities.

ARNTL: aryl hydrocarbon receptor nuclear translocator-like; ATG16L: autophagy related 16-like; B2M: β2 microglobulin; CAF: cancer-associated fibroblasts; FcγRIII: Fcγ receptor III; HLA: human leukocyte antigen; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; JAK–STAT: Janus kinase-signal transducer and activator of transcription; MEKi: mitogen-activated extracellular signal-regulated kinase inhibitor; MHC: major histocompatibility complex; NOD2: nucleotide binding oligomerisation domain-containing 2; NSAID: non-steroidal anti-inflammatory drugs; PD-L1: programmed death ligand-1; RANKL: receptor activator of nuclear factor κ-B ligand; ROS: reactive oxygen species; TCR: T-cell receptor; TLR: toll-like receptors; VEGF: vascular endothelial growth factor.

Adapted from Chen and Mellman,[11] used with permission.

The anti-idiotype therapeutic vaccine racotumomab has been conditionally approved in Latin America as maintenance therapy for advanced non-small cell lung cancer.[18] Recent breakthroughs in cancer immunotherapy demonstrate that clinical responses correlate with activation and expansion of tumour-specific T lymphocytes that mostly target mutation-based neo-antigens.[19] Due to their economically effective, cold chain transport and lack of harmful ingredients, many antigens are in development as plant-based vaccines, with only a few undertaking clinical trials in humans.[20,21]

The discovery that cancer cells may evade the response of tumour-reactive T cells has ignited efforts to improve the efficacy of antitumour immune responses, with the hope of removing limits on the activation and maintenance of T-cell effector function.

Table 1: Main types of cancer vaccines and adaptive immunotherapy.

Technology	Important consideration
Peptide-based vaccines[11,12]	Identification of peptide: natural, designed
Tumour cell vaccines (autologous or allogeneic)[13]	All relevant candidate antigens should be contained within cell
Recombinant viruses or bacteria with tumour antigens[14]	Delivery efficacy of antigen-encoding genes
Dendritic cell vaccines[15]	Choice of antigen in loading
DNA or RNA vaccines[16]	Easy delivery of multiple antigens with one immunisation
Anti-idiotype vaccines[17,18]	Choosing the right anti-idiotype
TACA mimetics[19]	Fidelity of mimicry
Neo-antigen vaccines[20-22]	Personalised

TACA: tumour-associated carbohydrate mimetics.

Reprogramming a tumour microenvironment to trigger T-cell activation and enhance tumour immunity, in effect, making a tumour hotter, provides insight into enhancing immune response. A strategy in support of this involves targeting the checkpoint's programmed cell-death protein-1 (PD-1) and programmed death ligand-1 (PD-L1) along with cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). Developed ICI are negative-regulators of T-cell immune function but with different mechanisms of action.[22]

PD-1 is expressed on activated T and B cells, natural killer T cells, Type 2 innate lymphoid cells (ILC-2), and myeloid cells.[23] CTLA-4 is expressed on Treg, activated T, and B cells. Expression of CTLA-4 on human natural killer T cells is unknown. As a result, CTLA-4 blockade disrupts the T-cell interaction with other antigen-presenting cells, such as dendritic cells, macrophages, or B cells, while anti-PD-1 blockade primarily blocks the tumour cell and cytotoxic CD8+ T-cell interaction.

Biomarker studies with anti-PD-1 and PD-L1, along with CTLA-4 clinical trials, support the hypothesis that these agents are most effective in patients who have pre-existing anticancer immunity. Determination of the basis of this pre-existing immunity may allow it to be utilised and amplified in vaccine strategies. ICI development demonstrated the concept of two nascent responses: first, the innate immune surveillance of cancer cells, and second, the adaptive immune response generated by the emerging tumour.

THE NASCENT IMMUNE SYSTEM

The immune system has evolved to distinguish self from non-self as a means to protect the host. A general feature of immune system mechanisms is that they detect structural features of non-self that mark them as distinct from host cells, reflecting a danger signal to the immune system.[24] The emergence of cancer[25] co-opts tissue-specific immune development to escape detection, augmented by failure of the immune system to perform its primary task of surveillance and elimination.[26] However, there is evidence that innate and adaptive surveillance does occur. Natural anti-carbohydrate antibodies are known to mediate cancer cell death.[27] Such natural antibodies, as part of innate immune surveillance, could promote tumour immunity by inducing immunogenic cell death, leading to immune priming and epitope spreading. Vaccine-induced anti-carbohydrate antibodies have displayed

the same antitumour characteristics as natural anti-glycan antibodies.[17] Thus, it may be possible to emulate innate antitumour responses in cancer therapies.

Burnet[28-30] and Thomas[31,32] hypothesised that the immune system can recognise nascent transformed cells, leading to elimination of the primary tumour formation.[33] Tumours that are not eliminated undergo a process of immune editing,[34] a reflection of the dynamic nature of immune surveillance, suggesting that at some point the antitumour immune surveillance was working, whether innate or adaptive. When the immune system is successful in eradicating incipient cancer cells, based on innate or adaptive immune response, no traces remain of its action. Mouse studies suggest that the immune system could initially see tumours as immunogenic, lending to a primed immune response.[35-37] After this initial phase, and if the cancer is not eliminated, the immune system shuts down, which leads to cancer escape from immune surveillance. As a result, the failure to eradicate cancer is not a failure of immune priming. These observations have led to the hypothesis that immune sculpting may result in the emergence of a less-immunogenic clone that is undetected by the immune system or downstream suppressive mechanisms. These downstream suppressive mechanisms include immune checkpoints that may allow malignant cells to evade an effectively-primed immune response, hence the rationale for the emergence of ICI.[22] It was 54 years following the research of Burnett and Thomas that anti-CTLA-4 therapies were approved.[38]

This leads to the question, where are we with enhancing the nascent response? Among clinical trials on clinicaltrials.gov, the search term "cancer vaccine" returned 58 early Phase I and combined Phase I/II, and 25 Phase II cancer vaccine trials that are actively recruiting. The distribution of these trials is listed in Table 2. The focus on T cells as the major immune effector mechanism for the action of cancer vaccines relies on processed tumour-directed peptides that activate T cells. This simplified view necessitates that, for acquired nascent immunity, the cancer-immunity cycle is initiated by the release of cancer cell antigens either shed by living cancer cells or released from dying tumour cells (see Figure 1). In either case, antigens are taken up and presented by antigen-presenting cells. Two antigen types are mainly represented in clinical trials in Table 2, with some considered as neo-antigens and others as tumour-associated antigens (TAA), incorporated into various platforms. Immune activity in cancer supports combining ICI with trials involving personalised tumour-specific neo-antigens and adaptive responses in general. However, currently there are 45 open vaccine trials without ICI, compared to 37 with ICI.

It is not clear whether cancer vaccines are part of a rational approach aimed at defined mechanisms. Systems vaccinology is an emerging field that applies omics technologies, in combination with bioinformatics tools such as transcriptional network analysis and predictive modelling, to study immune responses to vaccination.[39] This integration to vaccine design requires the understanding of the molecular network mobilised by vaccination. What are the global correlates of successful vaccination, beyond the specific immune response to the antigens administered, for understanding the mechanisms that underlie successful immunogenicity? Functional genomics are being used to analyse specific molecular signatures and antigens, for use as predictors of vaccination efficiency. The immune response to vaccination involves the coordinated induction of master transcription factors that leads to the development of a broad, polyfunctional, and persistent immune response, integrating all effector cells of the immune systems.

TUMOUR MUTATIONAL BURDEN, NEO-ANTIGENS, AND THE PERSONALISATION OF IMMUNOTHERAPY

Significant past research has focussed on genetic abnormalities affecting cancer-related genes (oncogenes and tumour suppressor genes), to define oncogenic drivers, and select the most important ones for therapeutic targeting. The remainder of the mutations discovered through genomic analyses were considered irrelevant. With the advent of next generation sequencing, a multitude of mutations were detected, leading the field to consider tumour mutation burden and discover neo-antigens. Research highlighting the role of the immune system led to the discovery

Table 2: Summary of open vaccine trials with and without checkpoint inhibitors.

Vaccine type	Early Phase I	Phase I	Phase I/II	Phase II
Neoantigen peptide	N/A	6 (1)	3	N/A
Cell-based	1 (3)	N/A	1 (1)	5 (4)
TAA-peptide	N/A	2 (8)	5 (2)	1 (5)
Vector-based	(2)	4 (11)	2 (1)	7 (7)

Number in parentheses reflects the number of trials without immune checkpoint inhibitors combination.
TAA: tumour-associated antigen.

that efficacy of ICI was correlated with tumour mutation burden and the presence of these neo-antigens.[40-44]

Neo-antigens are specific to hot tumours and may be unique to each patient. As a result, the anticancer vaccine effort has sought to develop specific reactive T cells to these neo-antigens. Their diversity, however, makes adaptation for immediate clinical use more difficult, requiring complicated platforms capable of rapid sequencing of the patient's genome to determine the most likely neo-antigens to be produced and given to the patient.[45-47]

The first step in the development of neo-antigen vaccines is the definition of the cancer mutanome for a specific patient.[48] The availability of high performance platforms for next generation sequencing allows the rapid identification of tumour mutations in comparison to matched healthy tissue samples. Alterations that are likely to result in immunologically meaningful mutations are single nucleotide variations, gene fusions, frame shifts by small insertions or deletions, and cancer-associated epigenetic aberrations[49] (at the transcriptional, translational, or post-translational levels). The second step is selection of the best neo-epitopes for vaccine design. Computational models have been developed to achieve this goal.[50-53] The third step is to select the format of delivery of the vaccine. Commonly used formats include long peptides and RNA. Others, such as DNA plasmids, engineered bacteria or viruses, and antigen-loaded dendritic cells, are under consideration.[48] The fourth step is to select the clinical setting for therapeutic application. Current practice suggests that these vaccines would work best in the adjuvant or minimal residual disease settings.

Some of these tumour-specific neo-antigens are known to be, or expected to be, common across a subset of patients and are called shared neo-antigens. Clinical trials of neo-antigens are shown in Table 3,[54-66] with some trials making use of typical peptide formulations while others involve a DNA or plasmid format. Neo-antigens such as tumour-specific antigens (TAA) are considered more immunogenic compared to self-antigens. TAA are now considered less favourable as vaccine candidates for several reasons: 1) being shared with normal tissues; 2) immune tolerance; and 3) heterogeneity within the same tissue, and among patients. However, heterogeneity may also occur with neo-antigens due to intratumour heterogeneity. Vaccines encoding xenoantigens, 'non-self' proteins that are highly homologous to their autologous counterparts, have been investigated as a means to increase immunogenicity and overcome tolerance to 'self' antigens.[67] Likewise, mimotopes or vaccines that incorporate peptide mimics of tumour antigens can function by eliciting increased numbers of T cells that cross react with the native tumour antigen.[68] Mimotopes, which are xenoantigens, can function like neo-antigens in inducing immune responses because they are different from self-antigens. Mimotopes have broad applications.

Table 3: Open clinical trials with neoantigen formulations.

Mode	Intervention	Phase	Cancer	Identification
Peptide	NeoVax Ipilimumab	I	Kidney	NCT02950766[54]
Peptide	GRT-C903 GRT-R904 Nivolumab Ipilimumab	I/II	Non-small cell lung, colorectal, pancreatic, shared neoantigen-positive solid tumours	NCT03953235[55]
Peptide	GRT-C901 GRT-R902 Nivolumab Ipilimumab	I/II	Non-small cell lung cancer, colorectal cancer, gastroesophageal adenocarcinoma, urothelial carcinoma	NCT03639714[56]
Peptide	Atezolizumab PGV-001 Poly ICLC	I	Urothelial/bladder cancer	NCT03359239[57]
Peptide	Personalised vaccine Pembrolizumab	I	Advanced cancer	NCT03568058[58]
Peptide	NEO-PV-01 Nivolumab Adjuvant APX005M Ipilimumab	I	Advanced melanoma	NCT03597282[59]
Peptide	ASV™ AGEN2017	I	Solid tumour (adult)	NCT03673020[60]
Peptide	RO7198457 Atezolizumab	I	Melanoma, non-small cell lung cancer, bladder cancer, colorectal cancer, triple-negative breast cancer, renal cancer, head and neck cancer, other solid cancers	NCT03289962[61]
Peptide	GEN-009 adjuvanted vaccine Nivolumab Pembrolizumab	I/II	Cutaneous melanoma, non-small cell lung cancer, squamous cell carcinoma of the head and neck/urothelial carcinoma, renal cell carcinoma	NCT03633110[62]
Neo-antigen vector	Personalised neo-antigen DNA vaccine	I	Pancreatic cancer	NCT03122106[63]
Vector	Durvalumab neo-antigen DNA vaccine	I	Triple-negative breast cancer	NCT03199040[64]
Vector	PROSTVAC-V PROSTVAC-F Nivolumab Ipilimumab Neo-antigen DNA vaccine	I	Metastatic hormone-sensitive prostate cancer	NCT03532217[65]
Vector	YE-NEO-001 Yeast	I	Colorectal cancer, breast cancer, head and neck squamous cell carcinoma, melanoma, non-small cell lung cancer, pancreatic cancer, liver cancer	NCT03552718[66]

Poly ICLC: polyinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose; YE-NEO-001: neoepitope yeast vaccine.

For example, one mimotope of tumour-associated carbohydrate antigens is currently undergoing clinical testing.[16,17] Mimotopes could be utilised to facilitate responses to cold tumours by recruiting TAA cross-reactive T cells and antibodies.

Presence of neo-antigens alone does not completely trigger an effective immune response; how the new antigens are presented also plays a role. For example, the presentation of a neo-antigen in low quantities, and with progressive minor modifications, may lead to immune tolerance rather than immune rejection.[69]

Conversely, high immunogenicity can still curtail a 'one size fits all' vaccine because of inherent heterogeneity in mutational rates. Neo-antigen vaccines are considered a means to enhance the nascent adaptive response, but to do so requires a vaccine to be developed from neo-antigens for every patient. This challenge may suggest an alternative strategy of using a whole-cell approach from a patient's own tumour, to customise or tailor a personalised vaccine.

CANCER METABOLIC STRESS AND RESISTANCE TO IMMUNOTHERAPY

Immune escape in cancer may occur in early stages of the immune response, as the cancer undergoes immune editing and becomes invisible to the immune system. Activating mutations of certain oncogenes (*KRAS*, *BRAF*, or *MAPK*) may result in decreased expression of major histocompatibility complex class-1 (MHC-I).[70,71] Alternatively, cancer may escape eradication at the later stages of the immune cycle, even after a vigorous effector T-cell response, by losing the ability to be destroyed (for example, by mutation of *CASP8*).[72] The cancer microenvironment is characterised by hypoxia and decreased availability of nutrients required for energy and cell structure maintenance, including glucose, lipids, and amino acids.[73,74] Anaerobic metabolism in the presence of oxygen (Warburg effect), a hallmark of cancer, leads to the production of large quantities of lactic acid that impair the function of immune cells. These metabolic changes lead to reprogramming of both the cancer cells and the immune cells in their microenvironment, resulting in a blunted immune response to the cancer and suppression of the effector CD8 T cells. Macrophage and myeloid cell differentiation is shifted to the immunosuppression type. Multiple therapeutic strategies have been proposed to overcome these obstacles.[73,75]

DEFINING PATIENT COHORTS: WHO BENEFITS?

The clinical experience with ICI has revealed that these drugs do not work for everyone; there are responders and non-responders, and only a minority of patients benefit.[76] ICI work in defined cohorts of patients, relating to levels of expression of the PD-1/PD-L1 axis, expression of mutated genes that lend to nascent responses, and those that have tumour-infiltrating lymphocytes and other immune cells (i.e., hot tumours). This contributes to complexity in determining patient cohorts for vaccine trials, which requires consideration of the vaccine therapeutic mechanism to enhance the immune response in hot or inflamed tumours (characterised by tumour-infiltrating lymphocytes), or alter the immune response in cold tumours to make them 'hot'. In addition, the PD-1/PD-L1 axis has the potential to be upregulated by transcriptional regulators that are yet to be defined but potentially associated with a cancer vaccine response. Some vaccines under consideration might have been dismissed because they upregulated transcriptional regulators known to shut down the immune response.

In the current era of immunotherapy, with the lack of definitive biomarkers, evaluation of tumours based on both their immune phenotype and genomic mutation profile may help determine which patients have a higher likelihood of responding to immunotherapies. Clinically, tumour burden reveals patient cohorts associated with therapeutic efficacy for cancer vaccines. Passively administered antibodies have been found to eliminate circulating tumour cells and systemic or intraperitoneal micrometastases in a variety of preclinical models; antibody-inducing vaccines may be beneficial in the adjuvant setting. Minimal residual disease is an indication for effective use of both monoclonal antibodies[77] and for cancer vaccines.[78] The results of the Keynote 522 Phase III clinical trial,[79] comparing chemotherapy with

pembrolizumab or placebo, revealed greater benefit in advanced stage of breast cancer than in early stage disease.

Insights and strategies from the immune foundation of ICI can be applied to the design and application of cancer vaccines, particularly to overcome the low antigenicity and heterogeneity of tumour-specific antigens. These include: 1) targeting multiple immunogenic antigens through polyvalent formulations;[80-82] 2) targeting a high fraction of tumour cells bearing each antigen, by considering the clonal nature of an antigen;[83] and 3) deriving cancer vaccines from the most immunogenic clonal antigen-loaded patients.[84] The majority of patients are not responsive to ICI because of the lack of tumour-specific effector cells. Consequently, cancer vaccines may be a means to elicit diverse antigen-specific effector cells.[85]

Different measures of antigen-specific tolerance or regulation may help predict immunological outcome from vaccination.[86] Santegoets et al.[87] demonstrated prolonged overall survival following treatment with a cancer vaccine (GVAX) in combination with ipilimumab in patients with advanced prostate cancer who had either: high pre-treatment frequencies of CD4+ CTLA-4+, CD4+ PD-1+, or differentiated (non-naïve) CD8+ T cells; or low pre-treatment frequencies of regulatory T cells or differentiated CD4+ T cells. These parameters suggest a highly immunocompetent patient. Such findings suggest that the identification of predictive biomarkers associated with long-term immune outcome could be beneficial for identifying patients most likely to benefit from antitumour vaccines. One measure of immunocompetency, for consideration as an inclusion criterion for cohort recruitment, is delayed-type hypersensitivity to recall antigens.[17] However, it has been suggested that this does not accurately reflect immune competence in patients with advanced- stage breast cancer, as research has demonstrated that patients who failed responses to recall antigens could still mount tumour-specific T-cell responses to a tumour antigen upon vaccination.[88]

Blank et al.[89] suggested the integration of all the parameters involved in the immune response into one dynamic framework; they called it the 'cancer immunogram.'[90] Seven variables are included in this model: tumour foreignness, the patient's general immune status, immune cell infiltration, checkpoints, soluble inhibitors, inhibitory tumour metabolism, and tumour sensitivity to immune effectors.

CONCLUSION

The ultimate goal of immunotherapy is to establish a durable population of highly active, tumour-specific responses that can lyse tumour cells and eradicate cancers. Evidence from various clinical trials that reflect the biology of immune response and cancer targeting lends to our understanding that cancer immunotherapy is a multifaceted strategy and that a single treatment modality will not suffice. The discovery of immune checkpoints and the success of their inhibitors has led to detailed investigation of the complicated interactions between different components of the immune system and microenvironment involved in the anticancer response.[91] A plethora of co-stimulatory pathways have been identified, with some now the subject of intense investigation to assess the benefit of their activation for augmenting the anticancer immune response. Other inhibitory pathways were identified and are being explored to assess their role in different cancers. This line of research has revealed the complexity of the immune landscape.

CTLA-4 and PD-1/PD-L1 appear to be the predominant immune checkpoints, but they are not the only ones. Different tumours may preferentially utilise particular inhibitory pathways. Eliciting an immune response through a tumour vaccine may also trigger these specific inhibitory pathways. Research understanding at this point assumes that vaccines that lead to the release of high concentrations of INF-γ are likely to induce the overexpression of PD-L1 on tumour cells, and may benefit from the combination of the vaccine with PD-1/PD-L1 inhibitors. If a vaccine were to increase the expression of GAL9/Tim3[92] or GITRL/GITR,[93] in addition to or instead of the PD-1/PD-L1 pathway, PD-1/PD-L1 inhibitors alone would be of limited use, as targeting the specific pathways triggered by the vaccine would be required. ICI rely on a primed nascent response. Cancer vaccines can provide priming and boosting of nascent responses but require ICI both to enhance a

response, in the case of CTL-4 therapy, and to block tumour suppression, as in the case of the PD-1/PD-L1 axis.

Tumours are heterogeneous in their antigenic make-up, and different antigens of the same tumour may have different immunogenicity. Some cancers succeed in evading the immune system by decreasing their foreignness; others lose their expression of MHC-I and become invisible to the immune system. The metabolic microenvironment of the tumour favours the reprogramming of immune cells to Type 2 responses. Tumours differ in their ways of shutting off the immune system using different immune checkpoints. Hosts are also heterogeneous in their ability to mount an immune response to tumour cells. Vaccines that utilise tumour antigens could potentially induce similar responses to their respective tumours and may trigger different inhibitory mechanisms.

There is insufficient clinical data to reveal a breakthrough in cancer vaccines, but a better understanding of the tumour microenvironment allows for consideration of new combinations. Current understanding has determined that certain vaccines increase the release of IFN-γ, which in turn increases the expression of PD-L1 on the tumour, leading to immune suppression that can be overcome with the use of ICI. Questions remain concerning the timing of treatments, adjuvants, immunisation routes, optimal immunogenic vaccines, tumour remodelling, and the cohort these combinations should be tested in.

A rational approach to the development of vaccine-ICI combinations would require detailed definition of the tumour antigenic immunogenicity, immunogenic heterogeneity, and the inhibitory mechanisms that the tumour uses to suppress the immune system; in effect, a systems-based immunology/vaccinology approach is needed. With various vaccine modalities and combinations to alter the cancer microenvironment and the immune response under research, personalised immunotherapy could be a reality in the near future.

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What's New

Successful Outcomes of Hepatitis C-infected Kidneys Transplanted into Healthy Recipients

WAITING times in the USA for kidney transplant can be as long as 5 years, with some patients waiting for 10 years or more. Recognising the importance of reducing these waiting periods, a research team from the University of Pennsylvania, Philadelphia, Pennsylvania, USA has found a new option for these patients.

Transplantation of a kidney from a donor who carried the hepatitis C virus (HCV) was previously considered too risky to transplant, which removed many potential donors from the register. Now, the first multicentre trial of HCV-infected kidney transplants into healthy recipients has shown the success of this approach, and the future possibilities for other organ transplantations.

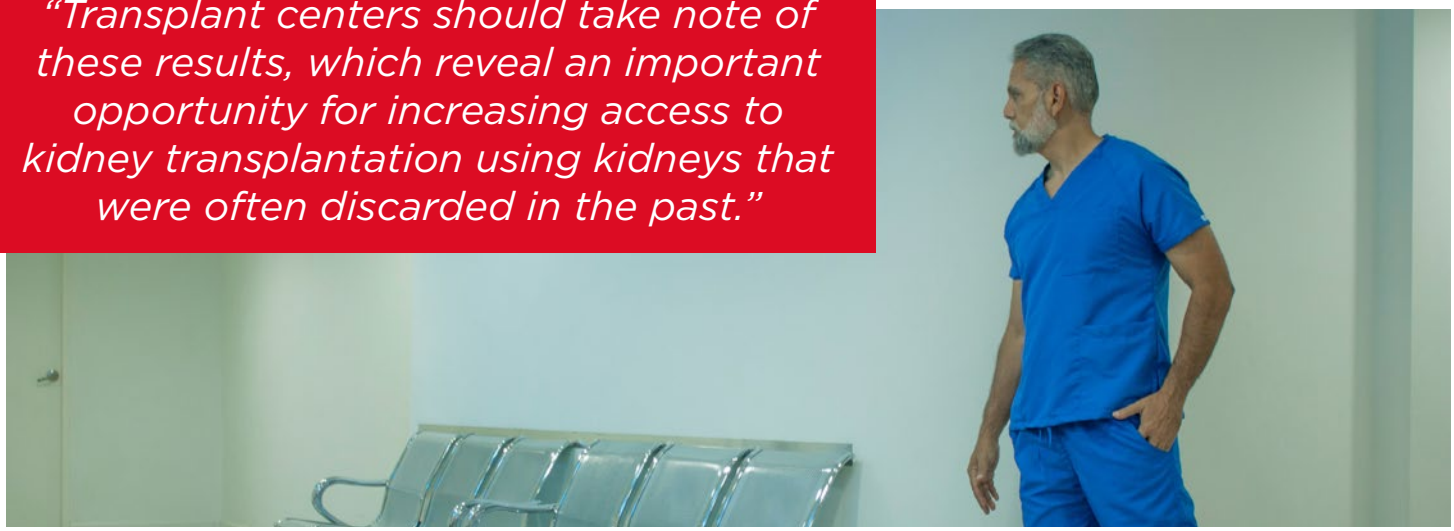
Across seven medical centres in the USA between May and October 2019, 30 HCV-positive kidney transplants took place, all with a high degree of success; after a 2-month course of glecaprevir/pibrentasvir (anti-HCV drugs), HCV was undetected in every single patient. Follow-up of the patients at 6 months also revealed that the kidneys were still functioning well, and no HCV genetic material was detected in patient blood.

Prof Peter Reese, senior author of the study, recommended that: "Transplant centers should take note of these results, which reveal an important opportunity for increasing access to kidney transplantation using kidneys that were often discarded in the past."

Though some serious adverse events occurred amongst the study patients, including one mortality 9 months after transplant caused by a bacterial infection, the researchers did not believe these were linked to HCV infection or the anti-HCV treatment.

The U.S. Centers for Disease Control and Prevention (CDC) estimated that in 2016 >2 million Americans were living with HCV infection. Considering this, the pool of donor kidneys will have the capacity to widen if HCV-infected kidneys are an option for transplantation. Prof Reese has high hopes for the technique: "Being able to make use of these HCV-positive kidneys from relatively young and otherwise healthy donors should improve current wait times for would-be recipients and has real potential to improve quality of life."

"Transplant centers should take note of these results, which reveal an important opportunity for increasing access to kidney transplantation using kidneys that were often discarded in the past."



Human iPS Cells Used to Reproduce Pathogenesis of Hereditary Polycystic Kidney Disease

IN VITRO studies using human induced pluripotent stem (iPS) cells by researchers at the Kumamoto University, Kumamoto, Japan, have successfully replicated the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD). This was the first documentation of collecting duct-derived cysts, which appear to be more closely linked to the pathogenesis of ADPKD than the previously used renal-tubule-derived cysts.

The gene editing CRISPR-Cas9 technology was used to produce the ADPKD cysts. Using the iPS cells, homozygous and heterozygous mutant versions of the *PKD1* gene, of which about 85% of all patients with ADPKD have the heterozygous mutation, were induced into renal tubules. The tubules were then treated with the cyst-exacerbating drug forskolin, and subsequent tubular cysts were reproduced, as well as tubule-derived mild cysts that were not genetically mutated. However, when the iPS cells were induced into collecting ducts and treated with forskolin, cysts only formed in the *PKD1* homozygous mutation.

Subsequent analysis by the team at Kumamoto University revealed that vasopressin, the antidiuretic hormone known to potentiate ADPKD cysts, only induced cysts in collecting duct cells containing the *PKD1* homozygous mutation. Interestingly, cysts formed in the collecting ducts of cells with the *PKD1* heterozygous mutation after administration of forskolin.

Prof Ryuichi Nishinakamura, the leader of the study, is hopeful for the implications of this study: "By analysing these collecting duct cysts, which are similar to actual clinical conditions, we may find mechanisms and develop new therapeutic methods that have been difficult to identify until now. We also expect that the replication of cysts from patient-derived iPS cells will lead to research and treatments for individual cases."

"By analysing these collecting duct cysts, which are similar to actual clinical conditions, we may find mechanisms and develop new therapeutic methods that have been difficult to identify until now."

What's New

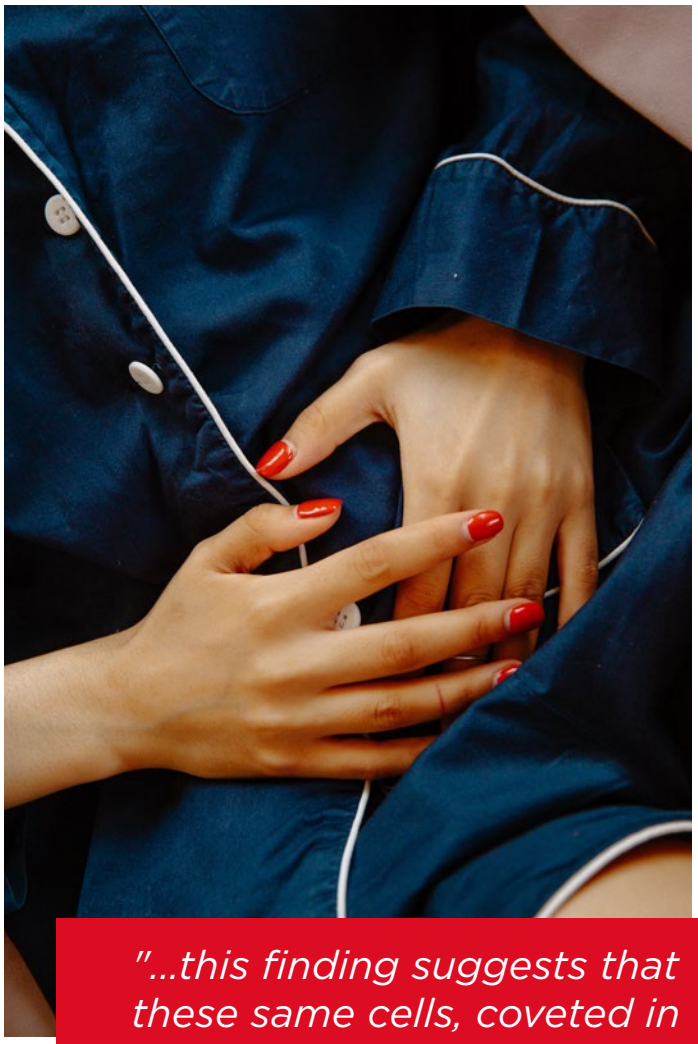
Memory T Cells May Be the Cause of Inflammatory Bowel Disease

INFLAMMATORY bowel disease (IBD), a group of intestinal disorders, cause a prolonged inflammation of the digestive tract and affect 6–8 million people worldwide. New research suggests that the lasting nature of IBD may be attributable to a long-lived immune cell causing persistent, damaging inflammation in the digestive tract.

Although various treatment options are available for IBD, many patients fail to respond long term, causing them to face chronic issues such as abdominal pain, cramps, and bloody stools. Genetic susceptibility, changes in the gut microbiome, and immune system dysfunction have been believed to play a role in IBD. Because the type of immune cells involved are unclear, Prof Gene W. Yeo, University of California San Diego School of Medicine, San Diego, California, USA, and his team collected samples from rectal biopsies or blood of patients with IBD and healthy controls and performed mRNA and antigen receptor sequencing.

It is well established that following an infection long-lived T cells, known as memory T cells, remain for rapid response upon pathogen re-exposure. In their findings the team discovered several subtypes of CD8+ tissue-resident memory T cells, one of which incorporated high levels of the transcription factor Eomesodermin and functioned to produce large quantities of cytokines and other molecules to kill newly detected infected cells. Conversely, excessive volumes of cytokines result in inflammation and tissue damage.

Results further showed that these inflammatory tissue-resident memory T cells were increasingly found in the intestinal tissues of patients with ulcerative colitis. Prof John T. Chang, University of California San Diego School of Medicine, commented that: "Long-lived memory cells are a goal of vaccines, but this finding suggests that these same cells, coveted in the fight against infectious diseases, may actually be harmful in the context of IBD." The researchers also found that these cells escape into the bloodstream and "this may explain why IBD can affect not just the intestines, but many other parts of the body as well." The findings might explain why IBD is chronic and present a possible future target for IBD treatment.



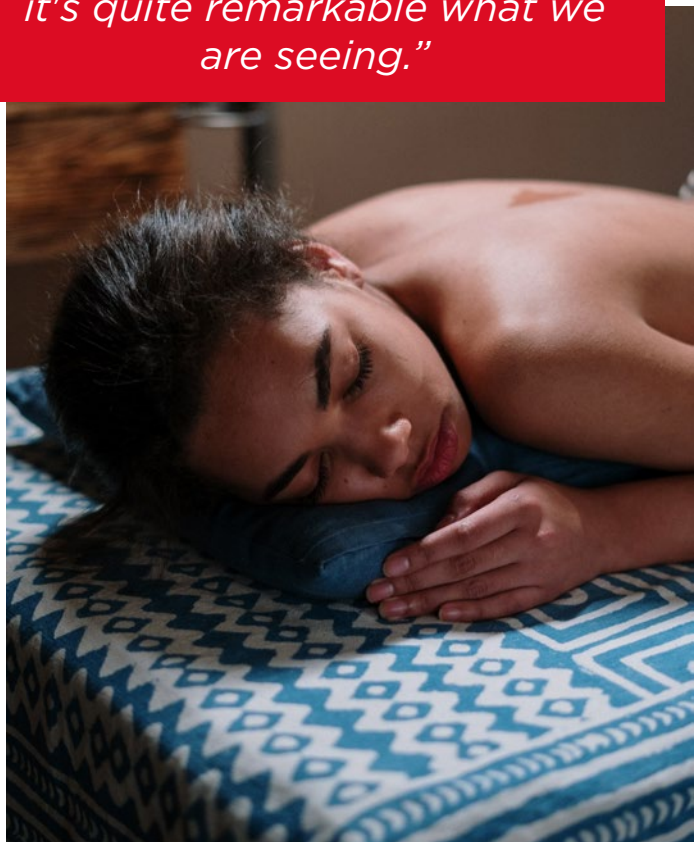
"...this finding suggests that these same cells, coveted in the fight against infectious diseases, may actually be harmful in the context of IBD."

Treating Faecal Incontinence with Repetitive Magnetic Stimulation

FAECAL incontinence, a debilitating problem affecting approximately 10% of the population, has been shown to be improved through magnetic stimulation of nerves that regulate muscles in the anus and rectum.

One-half of the patients report that current treatment strategies are unsatisfactory because they do not directly address the causes, such as nerve dysfunction in the anus and rectum. Therefore, Dr Satish Rao, director of Digestive Health Clinical Research Center, Augusta, Georgia, USA, and his team investigated the function of the nerves controlling those muscles.

"It's still in the early stage, but it's quite remarkable what we are seeing."



To analyse nerve activity, the team developed the painless translumbosacral anorectal magnetic stimulation test enabling the delivery of magnetic stimulation to nerves in the anus and rectum by placing a probe in the rectum and a coil on the back.

Results highlighted that nerve function was an issue in 80–90% of the patients tested, prompting the team to consider a similar approach where repetitive magnetic stimulation was applied externally to the back to aid healing of the nerves. The first study included 33 participants (average age: 60 years) who were treated with tanslumbosacral neuromodulation therapy (TNT) for 15 minutes to 1 hour, depending on the frequency applied. The 15-minute treatment, for example, included 15 stimulations per second (15 hertz). Responders were defined as those with at least a 50% reduction in weekly episodes of stool leakage.

Outcomes were beneficial for all treated; however, the low-frequency group with longer stimulation time benefited the most. Furthermore, the 1-hertz group had a 90% reduction in weekly episodes as well as significantly improved ability to sense a need to defecate and in their ability to hold more stool. Dr Rao positively commented: "It's still in the early stage, but it's quite remarkable what we are seeing."

Currently, Dr Rao is the project director and principal investigator of a lager study including 132 participants investigating how long the benefits of TNT last and how often follow-up sessions may be required. The current results are promising as TNT significantly shortened nerve to muscle activation time, with some patients reporting zero incontinence episodes following TNT.