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Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

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

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

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

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- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
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EMJ 3.2

EMJ Hepatology 6.1

This impressive eJournal marks the European Medical Journal's third specialist publication to be released this year, following the success of *EMJ Innovations 2.1* and *EMJ Urology 6.1*.

VIEW ALL JOURNALS ←

Welcome

I am honoured and delighted to welcome you all to the latest edition of our flagship publication: *EMJ 3.2.* As this year's second issue, this interdisciplinary journal draws upon the latest advances across a range of therapeutic areas and acts as a vital resource for medical professionals across the globe to learn and develop. Medicine is not a profession where disciplines work in isolation; communication and collaboration among specialities is vital to inspire innovation, share best practice, and ensure optimal patient outcomes. With this principle in mind, EMJ is proud to have compiled a hand-picked selection of peer-reviewed articles drawn from across our therapeutic areas. Haematology, gastroenterology, oncology, and urology are just some of the areas detailed in this edition of our flagship publication, so continue reading for comprehensive coverage of updates beyond your day-to-day specialism.

Our Editor's Pick for this edition was scribed by Macedo et al. to target the gap in the literature for new biomarkers in the management of gastric cancer. Dr Sorin T. Barbu, "Iuliu Haţieganu" University of Medicine and Pharmacy, Romania, one of *EMJ 3.2's* esteemed Editorial Board members, has commented that: "This paper opens new avenues for research into novel drugs targeting PKM2," so I am sure that it will provide food for thought.

Moving towards oncological insights, Yang et al. review the pathogenesis of multiple myeloma, including the hyperdiploid and nonhyperdiploid pathways, as well as the management strategies for this currently incurable malignancy, concluding that personalised treatment will be the future for these patients. In addition, Vavalà summarises a new era of therapy for non-small cell lung cancer, yet another disease with many unanswered questions regarding treatment and management. Other fascinating peer-reviewed articles within this edition include a review of overactive bladder in children by Oliveira et al., and insights into the diagnosis and staging of nonalcoholic fatty liver disease penned by Gallacher and McPherson; there truly is something for everyone to enjoy.

Before you delve into its pages, I would like to take this opportunity to thank all those who have contributed to *EMJ 3.2*, including authors and Editorial Board members, for making it a wonderful addition to our wide selection of available eJournals. I thoroughly hope you find the content as inspiring and insightful as we do!

Spencer Gore

Chief Executive Officer, European Medical Group

In indolent NHL…

As our knowledge evolves, so should our approach

Our understanding of indolent non-Hodgkin lymphoma (NHL), which includes follicular lymphoma (FL) and marginal zone lymphoma (MZL), has improved greatly. We know that indolent NHL is characterized in part by dysfunction within the tumor microenvironment that drives changes to the immune system.[1,2]

Celgene is researching approaches for patients, which seek to address the underlying immune dysfunction that contributes to these diseases.

Celgene is committed to researching chemotherapy-free approaches for patients with FL or MZL.

References: 1. Yang ZZ, Ansell SM. The tumor microenvironment in follicular lymphoma. *Clin Adv Hematol Oncol*. 2012;10(12):810-818. **2.** Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest*. 2012;122(10):3424-3431.

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Foreword

Dear colleagues and readers of the *European Medical Journal*,

It gives me great pleasure to welcome you all to *EMJ 3.2*, the second *EMJ* issue of 2018. This edition has an excellent selection of reviews and original research papers from different fields of medicine, which we hope will be a source of pleasure to individual readers.

The Editor's Pick was a difficult choice for me because all papers included in this issue are excellent, discussing relevant and challenging topics in their specialities. I finally opted for 'Pyruvate kinase and gastric cancer: A potential marker' by Macedo et al., a captivating review of this rather new topic, concluding that PKM2 or PDK1 measured from the blood or stools of patients, when analysed in combination with CA72-4, is a good marker for gastric cancer, a predictor of lower survival, and could help monitor treatment response to detect progression or relapse. The paper opens the field of research for new drugs targeting PKM2.

"The EMJ Editorial Board is delighted that you are joining us as readers and hope you will also join us as contributors."

The review on the management of non-small cell lung cancer by Vavalà summarises the role of immunotherapy and emphasises the current unanswered questions about biomarkers for treatment response, current treatments, and possible treatment combinations. Many other reviews are included, covering interesting topics like the role of phosphoinositide 3-kinase activity in the physiology of immature oocytes by Kim and Kurita, and modern methods for studying portal hypertension-associated angiogenesis in experimental research by Arefyev et al.

Inside *EMJ 3.2* readers can also find other fascinating, high quality papers, all of which were externally peer-reviewed. The *EMJ* Editorial Board is delighted that you are joining us as readers and hope you will also join us as contributors.

Sincerely yours,

Dr Sorin T. Barbu

"Iuliu Hatieganu" University of Medicine & Pharmacy, Romania

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+ Review of MEDICA 2017 Düsseldorf, Germany, 13th–16th November 2017
+ Review of the ILC 2018 Paris, France, 11th–15th April 2018

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+ A Transition from Disease-Centred to Goal-Directed Individualised Care of Patients with Multiple Morbidities: A Journey to Goal-Orientated Patient Healthcare Katarzyna Rygiel

EMJ Hepatology 6.1

+ Acute Fatty Liver of Pregnancy: Better Understanding of Pathogenesis and Earlier Clinical Recognition Results in Improved Maternal Outcomes Ashish Goel et al.
+ Targeting the Relaxin Pathway for Liver Disease Treatment Robert G. Bennett

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Plotting a Better Pathway for Parkinson's Disease: A New Composite Scale from My PD Journey

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A clinical scale that takes a broader, deeper view of Parkinson's disease (PD) symptoms is the latest milestone in a collaborative effort by My PD Journey, a multi-stakeholder initiative led by the European Parkinson's Disease Association (EPDA). My PD Journey aims to address challenges to timely, comprehensive, and individualised management of PD in Europe by measuring motor and non-motor symptoms.

Project leaders Prof Fabrizio Stocchi, San Raffaele University and Institute for Research and Medical Care, Rome, Italy, and Prof Pablo Martinez-Martin, Carlos III Institute of Health, Madrid, Spain, presented the aims and characteristics of the composite scale, as well as results from the first validation studies using the new model, to political stakeholders at a PD summit at the European Parliament on 18th February 2016. The results of the first validation study were published in January 2018 and concluded that the Parkinson's Disease Composite Scale (PDCS) appears to be a feasible, acceptable, reproducible, and valid scale for disease management.[1] A second and more extensive validation study, aimed at reaffirming the first study's findings, is currently underway. This paper will later outline the PDCS in more detail.

WHERE IT ALL BEGAN

The inspiration for the My PD Journey project developed from the widespread frustration in the PD community at the low levels of political awareness of PD as a health priority in Europe and the resulting absence or slow implementation of policies and initiatives to drive change at regional and national levels. For a disease that affects >1.2 million people across Europe,[2] a figure set to double by 2030 as the population ages,[3] the extent of this neglect is alarming. It is also costly: the annual financial burden of PD on the European economy has already reached at least €13.9 billion.[2] EPDA President, Knut-Johan Onarheim commented at a summit in February 2016 that these statistics "paint a bleak picture of the situation in Europe, especially considering the fact that the figures we do have are many years out of date." Mr Onarheim called for urgent collective action "on all fronts", with an emphasis on halting or slowing disease progression and improving PD patients' quality of life.

HURDLES TO DISEASE MANAGEMENT

People with PD face a number of hurdles to effective management of their disease, including inconsistent access to PD experts and delayed diagnosis. These delays can take

over a year; far longer than the maximum of 6 weeks recommended in clinical guidelines. The EPDA works to remove these hurdles by interacting closely with its member associations and engaging with European policymakers and other PD stakeholders. The EPDA also seeks to raise public awareness of PD as a key health challenge, support the development of national PD organisations across Europe, and eliminate the stigma and discrimination faced by people with the disease.

MY PD JOURNEY: A FIRST-OF-ITS-KIND MULTISTAKEHOLDER INITIATIVE

My PD Journey involves representatives from across the whole of the PD community, including umbrella European healthcare organisations, PD specialists, people with PD, carers, treatment industry companies, and members of the multidisciplinary healthcare teams managing the condition. The overarching objective is to create a sustainable environment in which people with PD have optimal and timely access to appropriate diagnosis, treatment, and care throughout the course of the disease. The scheme recognises their personal PD journey and the need for tailored disease management that will enable the patient to live as full a life as possible. My PD Journey has pioneered two flagship projects: pan-European research, completed in 2015, and the new PDCS.

THE EUROPEAN INVENTORY: GAINING A MORE ACCURATE INSIGHT INTO THE CHALLENGES FACING PATIENTS AND HEALTHCARE SYSTEMS

In November 2014, My PD Journey embarked on its first major phase of activity, the European inventory. This research project was a collaboration between the EPDA and the European Section of the International Parkinson and Movement Disorder Society (MDS-ES). As a result of this collaboration, care pathways for PD across Europe were assessed and compared with the goal of understanding the major hurdles to effective PD management, identifying gaps in existing care pathways, and finding examples of good national practice that could be replicated elsewhere.

Millbank Social Marketing Ltd., Cleveland, UK, was commissioned to conduct primary and secondary research for the project. This comprised a survey of 1,776 participants across 11 European countries, including people with PD, their carers, and healthcare professionals, followed by 194 in-depth interviews.[4]

The research was completed in 2015 and fed into recommendations for good practice in PD management at a national and European level that were presented at a landmark event in Brussels, Belgium, on 14th April 2015. Hosted by the Vice-President of the European Parliament, Mairead McGuinness, the workshop gathered stakeholders from across the entire PD community, as well as high-level representatives from the European Union (EU) institutions. These recommendations included:

- Timely diagnosis, access to specialised healthcare professionals, and continued management of the disease by a multidisciplinary team of experts.
- A personalised approach, reflecting the complexity of the disease and the crucial importance of tailoring treatment and care to individual needs and preferences.
- Patient and carer access to a PD healthcare professional trained to monitor and manage disease progression.
- Significant improvements in co-ordination and communication, particularly between primary and secondary healthcare professionals.
- Better PD training for healthcare professionals working in nursing homes and general hospital wards.
- All relevant information on the management and treatment of the disease should be available to people with PD and their carers.

POLICY RECOMMENDATIONS

The research findings from the European inventory also fed into a number of policy recommendations for EU and national member-state authorities, including EU support and funding for projects, such as My PD Journey; pooling of information and knowledge through reference networks; and support for further data collection to better inform diagnosis, treatment and care strategies. Since the event, the EPDA has engaged directly with influential EU

decision-makers in Brussels to advocate My PD Journey's policy recommendations and achieve concrete outcomes at a pan-European level. The political response to My PD Journey has been extremely positive, and European policymakers are starting to understand the challenge of PD. From these recommendations and meetings, the EPDA developed its EU political manifesto.

THE COMPOSITE SCALE: IMPROVING DIAGNOSIS AND DISEASE MANAGEMENT

The My PD Journey composite scale brings these efforts down to the practical level, with a tool to help doctors and people with PD tackle one of the earliest barriers to properly tailored and balanced management of the disease. It is designed to complement existing clinical scales, which the My PD Journey European Strategic Committee (ESC) felt could not measure and rate PD symptoms in a way that reflects the full complexity and scope of the condition.

The effects of PD on motor functions are generally well recognised, including tremors, rigidity, bradykinesia, and postural instability. However, there is less awareness of non-motor symptoms, such as depression, anxiety, sexual dysfunction, constipation, or urinary problems, and the magnitude of their physical, psychological, and cognitive impact on patients, families, carers, and healthcare systems.

The full breadth of PD symptoms may be difficult to express, capture, and track within the confines of a 10–15-minute doctor's appointment. The situation is aggravated by the present need to use several rating scales, such as UPDRS, NMS, UDysRS, mAIMS, and MoCa, when assessing disease severity. Not only are standard assessments designed for other conditions and not sensitive enough to give a full picture of PD, but health and regulatory authorities now demand objective measures when allocating budgets for different diseases. As things stand, a straightforward holistic scale to evaluate the status of PD patients that takes into account both motor and non-motor symptoms, as well as treatment complications and disability level, does not exist. The lack of such a scale represents a significant obstacle to timely and appropriate interventions that will optimise the management of PD.

PARKINSON'S DISEASE COMPOSITE SCALE

The development of such a straightforward, holistic scale was the thinking behind the PDCS, a project spearheaded by Prof Fabrizio Stocchi, and Prof Pablo Martinez-Martin.

The aim was to develop a new, easy-to-use clinical scale that would not replace, but work in concert with, existing instruments. These latter scales remain crucial to the precise assessment of certain PD symptoms (e.g., NMS for non-motor symptoms and UDysRS for dyskinesia). At the same time, the PDCS would provide a more comprehensive overview of patient status, including quality of life considerations and the relevance of particular symptoms to healthcare systems as well as patients themselves. For example, tremors may be a significant feature of PD for patients in terms of day-to-day activities, self-confidence, and stigmatisation. They may be less of an issue for healthcare systems with respect to disease management and associated costs. By the same token, a patient falling down can have a substantial impact from both perspectives.

The PDCS was designed to incorporate the most important motor, non-motor, and treatment-complication symptoms of PD. These are symptoms identified through expert experience and patient reports as more significant in determining disease severity. The composite scale also tried to give different weight to different symptoms, according to their impact on quality of life. PDCS also needed to be straightforward enough to be understood by all healthcare professionals addressing PD, as well as by PD patients themselves.

Developing the Scale

Initial work on the PDCS began in September 2014. A pilot study involving 70 patients with mild-to-moderate PD produced satisfactory outcomes in terms of the tool's acceptability and hypotheses-testing.[1] These tests highlighted some problems of internal consistency that were further assessed in the first validation

study of the composite scale. This multicentre study involved a total of 194 PD patients, with a mean age of 66.51 years, in five countries (Australia, Italy, Romania, Sweden, and the UK). It looked at:

- Feasibility and acceptability: The extent to which the PDCS could be used successfully in a clinical setting.
- Reliability (internal consistency and stability): How much the scale was free from random errors.
- Validity (hypothesis-testing validation): The extent to which the scale assessed the underlying theoretical construct it was designed to measure.
- Precision: The scale's ability to distinguish between small differences in symptoms.

The validation study indicated that the PDCS was a feasible, acceptable, reproducible, valid, and precise instrument for more holistic measurement of PD symptoms.

Given the pragmatic characteristics of the scale, the EPDA and the scale's developers proposed a second, larger validation study to confirm the initial findings and promote a broad use of the PDCS. This second study, due to be finalised and published in 2018, is being co-ordinated by Fabiana Giada Radicati at the IRCSS San Raffaele, Rome, Italy. There are 22 centres participating in the second validation study from 14 countries, involving around 700 patients. The My PD Journey team is currently working with neurological and clinical bodies, policymakers, and patient organisations to roll out this new tool across Europe, and beyond.

THE JOURNEY CONTINUES

My PD Journey will continue to work with stakeholders at all levels of PD diagnosis and management to improve patient outcomes by designing, implementing, evaluating, and optimising patient-centred care models for this life-changing disease. My PD Journey strives to publish all the European inventory research and composite scale studies in peer-reviewed journals, while exploring options for further data collection and analysis and ensuring the roll-out of the composite scale in clinical and patient settings across Europe.

With the support and encouragement from healthcare professionals, research funders, PD experts, policy makers, and the general public, the coalition can leverage the benefits of collaborative efforts across national, cultural, and professional boundaries, to ensure that living with PD means a life both lived to the full and integrated as much as possible with healthcare practices and society as a whole.

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- The convenience of once-daily administration with food[8]

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*Modified intent-to-treat population: population comprising Ph+ patients with typical BCR-ABL1 transcript types (e13a2 and/or e14a2).

Abbreviations CML, chronic myeloid leukaemia; CP, chronic phase; ELN, European LeukaemiaNet; MMR, major molecular response, ≤0.1% BCR-ABL1 transcripts on the international scale with ≥3,000 ABL1 assessed. MR4.5, ≤0.0032% BCR-ABL1 transcripts on the international scale with ≥30,990 ABL1 assessed; Ph+, Philadelphia chromosome-postive; TEAE, treatment-emergent adverse event.

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Delivering Precision Medicine and Patient-Centred Care Through a Multidisciplinary Approach

This symposium took place on 15th February 2018 as part of the 13th Congress of European Crohn's and Colitis Organisation (ECCO) in Vienna, Austria

Chairperson: Krisztina Gecse[1]

Speakers: Claudio Fiocchi,[1] Krisztina Gecse,[2] Antonino Spinelli,[3] Frank Behrens,[4] Luisa Avedano[5]

1. Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio, USA
2. Academic Medical Center, Amsterdam, Netherlands
3. Department of Biomedical Sciences, Division of Colon and Rectal Surgery, Humanitas University, Milan, Italy
4. Centre of Innovative Diagnostics and Therapeutic Rheumatology/Immunology, Frankfurt, Germany
5. European Federation of Crohn's and Ulcerative Colitis Associations, Brussels, Belgium

Disclosure: Dr Gecse has received consultancy fees and/or speaker's honoraria from Amgen, AbbVie, Boehringer Ingelheim, Ferring, Hospira, MSD, Pfizer, Sandoz, Takeda, and Tigenix. Prof Fiocchi has received research support, fees, or non-financial support from the National Institute of Health, Ferring, MSD, Janssen, UCB, Sandoz, KU Leuven, Canada Future Directions, and the State Institute of Coloproctology, Moscow, Russia. Prof Spinelli has received consultancy fees and/or speaker's honoraria from Ethicon, Tigenix, and Sandoz. Dr Behrens has received research support from AbbVie, Pfizer, Roche, Chugai, Prophylix, Bioline, and Novartis, and has received consultancy fees and/or advisory board honoraria from AbbVie, Pfizer, Roche, Chugai, UCB, BMS, Celgene, MSD, Novartis, Biotest, Janssen, Genzyme, and Lilly. On behalf of EFCCA, Dr Avedano has received consultancy fees, speaker's honoraria, and funding from Vifor, Pfizer, Celltrion, Samsung, Sandoz, and Shire.

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Meeting Summary

The current treatment strategy for patients with inflammatory bowel disease (IBD) aims to enable physicians to deliver optimal care and to improve the role that patients play in treatment decisions. The multidisciplinary team (MDT) approach integrates the patient's perspective and sees the discussion of treatment options with both gastroenterologists and surgeons as early as possible. The MDT approach is also vital in managing the risk of IBD and cardiovascular-related comorbidities in patients with psoriasis (PsO) and psoriatic arthritis (PsA), where selection of appropriate medication may affect both the rheumatic condition and the associated comorbidity. Close interdisciplinary interactions between gastroenterologists, rheumatologists, and/or dermatologists are vital, and the ensuing knowledge transfer facilitates the provision of optimal patient care.

Personalised medicine will have a profound impact on future treatment algorithms in IBD and other chronic inflammatory conditions. Owing to the complexity of these diseases, a novel approach is urgently needed that will aggregate data from multiple systems and integrate it into a so-called 'IBD interactome'. This may help identify and target the key molecular components responsible for inflammation. Future treatment practices will also address the psychosocial aspects of IBD by empowering patients and integrating their perspective into the shared treatment decision-making process early on.

Optimising Multidisciplinary Care in Inflammatory Bowel Disease

Doctor Krisztina Gecse and Professor Antonino Spinelli

In an MDT approach to the management of IBD, the treatment plan is overseen by gastroenterologists and surgeons, with additional input from other MDT members including psychologists, stoma nurses, dermatologists, rheumatologists, IBD nurses, pathologists, and radiologists (Figure 1). Early involvement of the surgeon is particularly important in cases of acute severe ulcerative colitis (UC), a severe flare, experienced by approximately 20–25% of patients with UC, that requires hospitalisation and immediate intensive medical and surgical monitoring.[1-4]

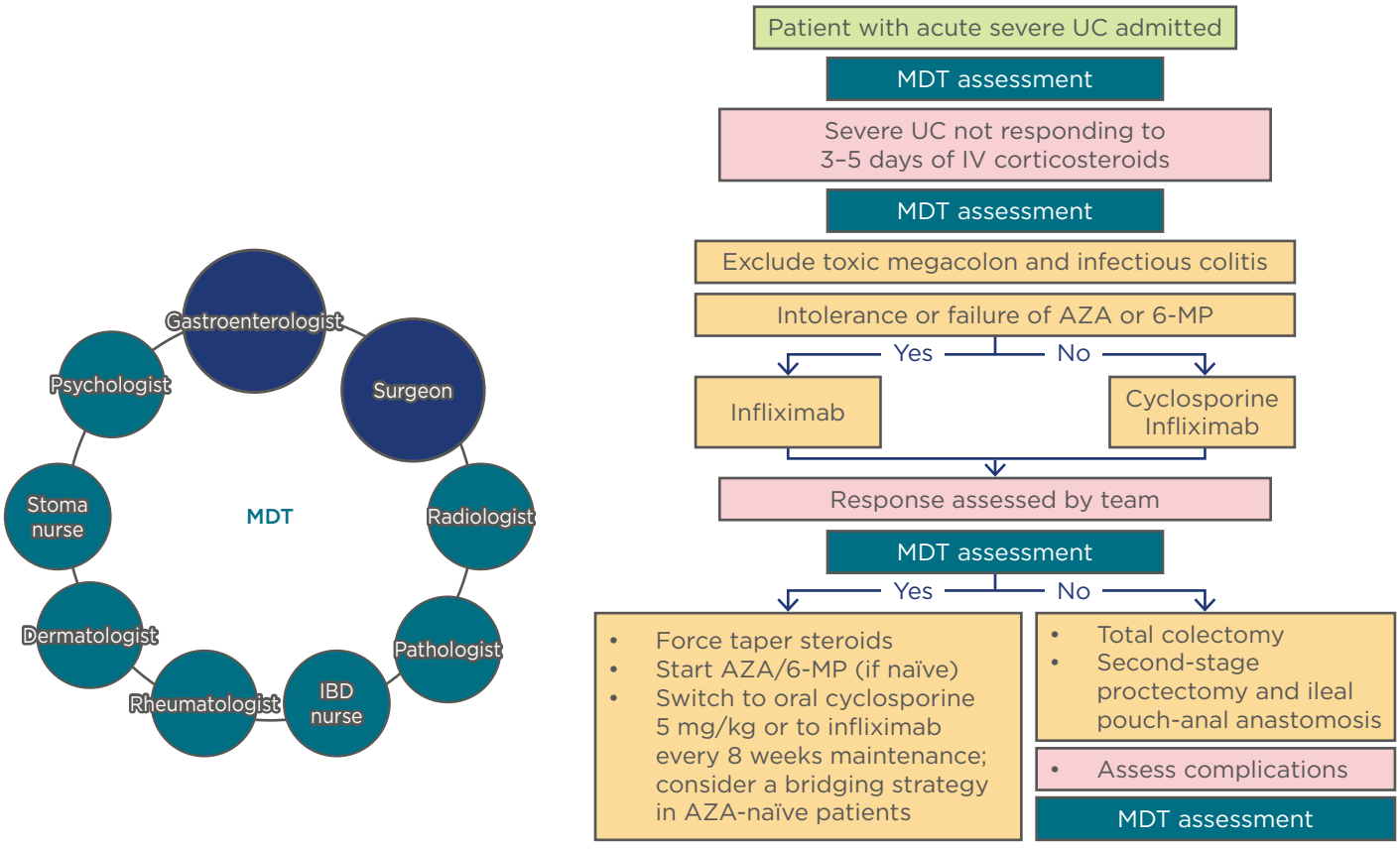

Figure 1: Multidisciplinary team involvement in treatment decisions for acute severe ulcerative colitis.

Gastroenterologists and surgeons comprise the core of the MDT, which also includes the IBD nurse, psychologist, rheumatologist, stoma nurse, dermatologist, pathologist, and radiologist.

AZA: azathioprine; IBD: inflammatory bowel disease; IV: intravenous; MDT: multidisciplinary team; MP: mercaptopurine; UC: ulcerative colitis.

Adapted from van Assche et al.[5]

Currently, the typically applied definition of acute severe UC follows the Truelove and Witts criteria: frequent bloody diarrhoea (≥6 times a day) accompanied by signs of systemic toxicity, such as pulse rate >90/minute, body temperature >37.8°C, haemoglobin <105 g/L, erythrocyte sedimentation rate >30 mm/h, or C-reactive protein >30 mg/L.[6] After admission, a daily clinical assessment is performed to facilitate early recognition of complications and negative prognostic factors which help predict the need for surgical intervention. Surgery becomes mandatory if complications occur, such as toxic megacolon, perforation, or severe colorectal haemorrhage.[7]

First-line rescue therapy with a 5-day intensive intravenous regimen of steroids has a remission rate of 73%.[2] However, after 3 days of intensive treatment, patients with either frequent stools (>8/day), and/or elevated C-reactive protein (>45 mg/L) need to be identified for further intervention. Failure of first-line and subsequent rescue therapies increases the risk that surgery will be required.[8] The likelihood of colectomy following the initial rescue therapy has been analysed in patients undergoing second-line rescue treatment with cyclosporine A or infliximab.[9] In this study, no difference was found between the two regimens; however, another retrospective study has shown that accelerated dosing of infliximab results in a significantly lower colectomy risk compared with standard dosing.[9,10] Surgery is indicated in most patients who do not respond to standard rescue treatments. Daily clinical assessment is performed after the initiation of therapy. The timing of surgical intervention is critical because prolonged, ineffective systemic treatment can lead to a deterioration in general health and contribute to postoperative morbidity and mortality.[7]

It is important to conduct a surgical consultation promptly after patient admission and to intimately involve the patient in this consultation and subsequent treatment decisions, as this helps to manage patient expectations. In the setting of acute UC, patients should be informed about available medical and surgical options and their respective prognoses as early as possible. The decision regarding surgery timing should be shared by both gastroenterologists and colorectal surgeons.[7,11] Colectomy can and should be considered as the first option in certain subsets of patients, such as when the patient has a contraindication to steroids and/or biologics, when complications arise (e.g., toxic megacolon or perforation), or if there have been recurrent hospitalisations for acute severe UC. In addition, patient age may be a factor in selecting the optimal treatment approach.[7]

Delaying surgery for patients who fail to respond to rescue therapy is associated with an increased risk of postoperative complications. A prospective study of 80 patients undergoing subtotal colectomy for acute severe UC in the UK between 1994 and 2000 showed that a prolonged preoperative hospital stay is associated with higher short and long-term postoperative morbidity.[12] Similarly, a retrospective analysis of a nationwide database from the USA included 7,108 patients who underwent subtotal colectomy from 1995–2005.[13] In this study, prolonged preoperative hospital stay was associated with both higher postoperative morbidity and mortality.[13]

The recommended surgical procedures in acute UC are colectomy and ileostomy, with the rectum left *in situ*.[11,14] A laparoscopic approach is at least as safe as open surgery, but it has the advantages of a decreased rate of postoperative infections and abdominal abscesses and is associated with a shorter hospital stay. It is also associated with a better-preserved body image and reduces the risk of lower bowel obstruction.[7,11]

In conclusion, there are several time points at which an MDT meeting should be part of the treatment decision process (Figure 1). Gastroenterologists and surgeons should be consulted as soon as the patient with acute severe UC is admitted to the hospital; further meetings may be required after first-line rescue treatment or on Day 3 of steroid treatment, and when assessing the response to second-line rescue treatment.[5] Any complications that arise should be assessed by the whole MDT, and, if necessary, post-second-line daily evaluations by the gastroenterology team should also include daily consultations with the surgical team. In conclusion, the patient should be included in setting treatment goals in UC as

well as other IBD indications. Furthermore, the patient's quality of life after surgery should be taken into account when assessing treatment success.

Managing Comorbidities: Lessons Learned from Rheumatology

Doctor Frank Behrens

In rheumatologic diseases, associated diseases and comorbid conditions occur in parallel with the core disease, and may drive treatment decisions and necessitate a MDT approach to management. The number of surgical interventions for rheumatic diseases, including rheumatoid arthritis, has reduced dramatically since the introduction of biologics to the treatment landscape.[15] With the increasing number of therapy options currently available to patients with arthritis, the physician needs to carefully choose interventions that will not only address the signs and symptoms of arthritis, but also treat any associated disease.

PsO is one of the most common skin diseases in Central Europe and 30% of patients with PsO are also affected by PsA.[16] PsA is associated with structural damage to the joints, as well as with systemic inflammation. The disease is highly heterogeneous, and symptoms may include swollen, tender joints, enthesitis, ankylosis, nail PsO or nail impairment, and skin manifestations.[17] In addition, patients with PsO are at risk of developing IBD and often have cardiovascular and metabolic comorbidities.

A nationwide register study in Denmark showed that patients with PsO had a higher incidence of Crohn's disease (CD) compared with the general population. Adjusted incidence risk ratios were 1.28 (95% confidence interval [CI]: 1.03–1.59) for mild PsO, 2.56 (95% CI: 1.87–3.50) for severe PsO, and 3.42 (95% CI: 2.36–4.95) for PsO with concomitant PsA. Similarly, the adjusted incidence risk ratios of UC were 1.49 (95% CI: 1.32–1.68), 1.56 (95% CI: 1.22–2.00), and 2.43 (95% CI: 1.86–3.17), respectively.[18] Two prospective Nurse's Health Studies of 174,476 women in the USA showed an 8-fold increased risk of CD in women with PsO and PsA (Table 1).[19] It should be noted that the presence of concomitant PsA increases the risk of IBD >3-fold and a large proportion of patients with spondyloarthropathies (PsA, ankylosing spondylitis, reactive arthritis) have inflammatory lesions of the bowel that may not be typical of IBD, but are indicative of chronic inflammation.[20,21] Notably, although the occurrence of intestinal inflammation has been linked with the use of certain medications (e.g., corticosteroids), other studies suggest that it is caused by the rheumatic condition itself.[22]

Awareness of possible inflammatory comorbidities should be maintained when making treatment decisions. For example, therapies targeting interleukin-17 have demonstrated efficacy in PsO, but not in IBD, whereas anti-tumour necrosis factor (TNF) therapies have shown activity in both PsO and IBD.[23-25] Thus, if a patient with PsO shows signs of inflammation in the gut that may indicate early stages of IBD, an anti-TNF agent may be more appropriate.

Psoriatic disease is also associated with an increased risk of cardiovascular disease. Increased incidence of major adverse cardiac events, myocardial infarction, cerebrovascular disease, atherosclerosis, and ischaemic heart disease in patients with PsO results in a reduced life expectancy compared with the general population.[26-28] A recent meta-analysis of five studies, totalling 49,795 patients with PsO, demonstrated that some of the available treatments for PsO may reduce the risk of adverse cardiovascular events.[29] Specifically, treatment with TNF inhibitors was associated with a significantly lower risk of cardiovascular events compared with topical or phototherapy (relative risk: 0.58; 95% CI: 0.43–0.77; p<0.001) or with methotrexate treatment (relative risk: 0.67; 95% CI: 0.52–0.88; p=0.003).[29] The new European guidelines on the treatment of rheumatic conditions warn clinicians of the higher risk of cardiovascular disease in patients with rheumatoid arthritis and other inflammatory joint diseases and place the responsibility for cardiovascular risk management on the rheumatologist.[30]

The Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) has recently reviewed the data on the most pertinent comorbidities in patients with PsO or

PsA and their effect on treatment, and provided detailed treatment recommendations.[31] In line with these recommendations, individual treatment decisions should be driven by available efficacy data, both in the core disease and in possible concomitant conditions; concomitant conditions can be identified by a careful assessment of symptoms beyond those associated with the core disease. Increasing the predicted lifespan for patients with chronic rheumatic diseases to equal that of the general population and beyond should be used as the measure of treatment success.

Table 1: Patients with psoriasis have a significantly increased risk of developing Crohn's disease.

Patients with Crohn's disease (cases; PY)	Age-adjusted RR (95% CI)	Multivariate-adjusted RR (95% CI)
No psoriasis (174; 2,401,946)	1.00	1.00
Psoriasis (11; 41,960)	3.74 (2.03–6.89)	3.49 (1.89–6.44)
Psoriasis with psoriatic arthritis (3; 5,661)	7.99 (2.55–25.08)	6.54 (2.07–20.65)

Results of a prospective cohort study from the USA (N=174,476) indicate that women with PsO with or without PsA have a significantly increased risk of developing Crohn's disease compared with the general population.

CI: confidence interval; PsA: psoriatic arthritis; PsO, psoriasis; PY: patient years; RR: relative risk.

Adapted from Li et al.[19]

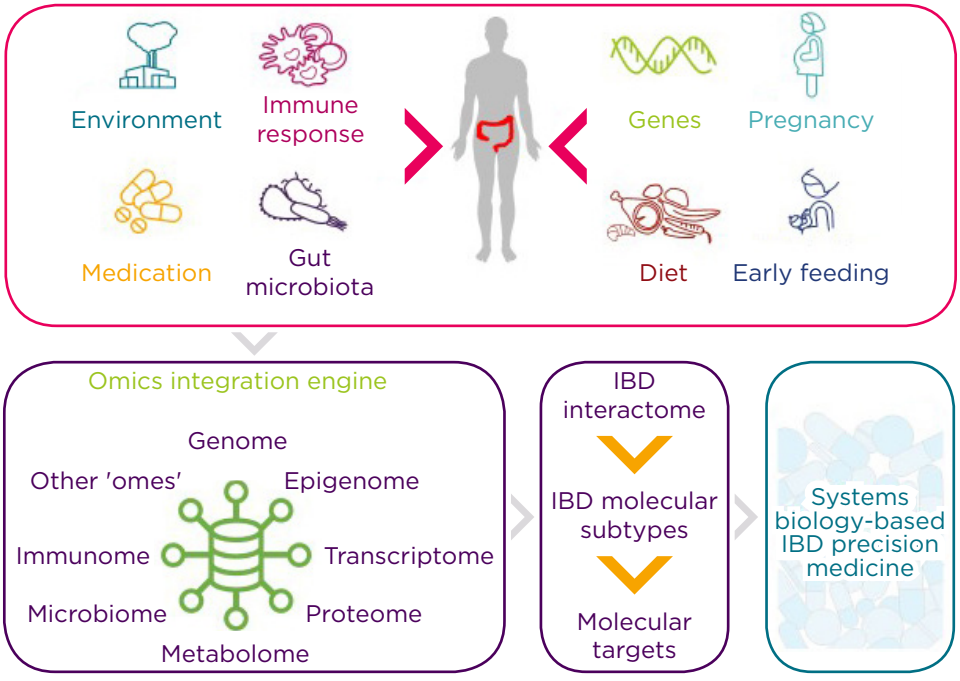

Figure 2: New multidisciplinary approaches to inflammatory bowel disease.

Future systems biology approaches will integrate data obtained through analysis of genome, epigenome, etc., producing an overall disease network. Other 'omes' are used in the sense that there might be still unrecognised omes that are relevant for IBD.

IBD: inflammatory bowel disease.

Adapted from Stern et al.,[39] *Ananthakrishnan et al.,*[40] *and Schultze.*[41]

New Multidisciplinary Approaches: What Does the Future Look Like?

Professor Claudio Fiocchi

The last two decades have been spent in constant search for better treatments for immune-mediated inflammatory disorders; however, rather than achieving a cure, we have merely increased the number of therapeutic options available. New therapies are designed based on increasing knowledge of disease pathogenesis and aimed against carefully selected targets, including molecules that regulate interactions between leukocytes and endothelial cells at sites of inflammation, molecules that modulate the migration of T cells in the blood, and intracellular signalling components, such as Janus kinases.[32,33]

The introduction of anti-TNF therapies revolutionised the management of several autoimmune and chronic inflammatory diseases: rheumatoid arthritis, PsA, PsO, ankylosing spondylitis, CD, and UC. Nevertheless, up to 40% of patients do not respond to initial treatment with biologics (infliximab, adalimumab, golimumab, vedolizumab, tofacitinib), and up to 50% experience reduced responses over time.[25,34] Furthermore, of the biologic therapies that have been carefully designed in past decades, many failed to work in the clinic.[35] The community seems to have reached a plateau of options and may be at the stage where creating a new drug based on current understanding is not the best way forward; instead, future drug discovery should be based on comprehensive systems biology approaches.[36]

Chronic inflammatory diseases of unknown aetiology are highly complex and are often driven by a combination of factors. Intestinal inflammation in IBD involves an interplay between various subtypes of immune and non-immune cells, and may be influenced by the composition of gut microbiota and other environmental factors, such as diet and smoking (Figure 2).[35] It is known that the exposome (external environment), genome (patient's genetic material), immunome (pattern of immune response), and microbiome (composition of gut microbiota) influence each other in many positive and negative ways, and no two patients are alike.[37] Previous studies have generated a wealth of genomic, epigenomic, transcriptomic, proteomic, metabolomic, and microbiome data, which may be used in future 'network medicine' initiatives.[38] These systems biology approaches will involve studying the molecular interactions between each of the omes, producing an overall disease network: the so-called 'IBD interactome'. New concepts and tools are needed to define this IBD interactome, build a comprehensive molecular map of IBD, and identify the key molecular drivers of inflammation.[35]

The number of possible molecular interactions in healthy and diseased tissues is immense, and big data integration methods need to be developed to handle this information effectively.[42] In addition, data obtained from single tissues may miss important regulatory interactions that are responsible for the pathogenesis of IBD. To reproduce this complexity of IBD, biological omics data derived from multiple sources will need to be integrated to construct regulatory network models.[43] Novel software packages are available to assist with the modelling and visual display of complex omics data, including Cytoscape, VisANT, Pathway Studio, ProViz, and others.[35] Further optimised bioinformatic tools are continuously being developed with the aim of simplifying the identification of central hubs (central regulatory molecules) within these extensive datasets.

Identifying the key components of the 'IBD interactome' will improve the specificity of the approach to disease subtyping, biomarker discovery, and drug repurposing. Importantly, it will enable a molecular classification of patients that is essential for the development of individualised treatment algorithms and for optimising the efficacy of existing treatment options.

Panel Discussion Precision Medicine: Current and Future Considerations

Professor Claudio Fiocchi, Doctor Krisztina Gecse, Professor Antonino Spinelli, Doctor Frank Behrens, Doctor Luisa Avedano

A panel discussion followed the presentations, allowing the faculty to share insights into current and future considerations for treating IBD, from the perspectives of the patient, gastroenterologist, colorectal surgeon, and rheumatologist.

Considering unmet needs from the patient's perspective, Dr Avedano, CEO of the European Federation of Crohn's & Ulcerative Colitis Associations (EFCCA), explained that it is important for patients with IBD to achieve a better balance between quality of care and quality of life. Sometimes, an excellent approach to the disease itself does not offer the improvement that the patient had hoped for. Additionally, IBD is a complex chronic disease that has a large psychosocial component. Often, the diagnosis of IBD has an enormous psychological impact on the patient, and physicians need to adopt a step-by-step approach to providing the patient with the right information at the right time, giving the patient time to assimilate the information to prepare for treatment discussions.

In this setting of chronic disease, the role of IBD nurse should not be underestimated. Dr Avedano mentioned that many nurses play the role of psychologist and offer advice on anxiety management and nutrition. In the context of interdisciplinary case management, MDT consultations may also help to decrease the patient's anxiety. Different specialists often express slightly different views, and the possibility to speak to the whole team at once may bring patients on board faster and prevent confusion.

Dr Gecse and Prof Spinelli emphasised the importance of shared decision-making and involving the patient as early as possible, especially when raising the potential need for surgery, which may be harder for the patient to accept than non-invasive treatment options. Prof Spinelli noted that precision surgery in IBD can and should follow an individualised, patient-centric approach. The future of IBD surgery is to achieve clinical effectiveness while preserving the patient's body image and quality of life.

Another aspect of individualised care lies in the variety of clinical presentations and molecular profiles of chronic immune-mediated inflammatory diseases. Prof Fiocchi emphasised that the same therapy or environmental factors may affect individual patients differently. Furthermore, diseases evolve on the molecular level, causing changes that may alter treatment efficacy. Dr Behrens remarked that many observations can be extrapolated across different inflammatory conditions; for example, the impact of environmental factors and microbiota observed in the pathogenesis of IBD may also be applicable to rheumatic diseases.

Conclusion

In conclusion, a personalised, patient-centric model will shape the future of IBD care, and a MDT approach will contribute to the clinical success of new therapies. The future treatment landscape will incorporate knowledge derived from integrating data in a systems biology approach. Finally, patient empowerment should be addressed by involving patients directly in treatment decision-making and by addressing psychosocial aspects of IBD early on.

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Treating Psoriasis: What Is New About Fumaric Acid Esters?

This oral presentation took place on 7th April 2018, as part of Almirall's 11th Skin Academy meeting in Barcelona, Spain

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Meeting Summary

This publication covers the first session of Almirall's 11th Skin Academy meeting in Barcelona, Spain. This year, the meeting theme was 'The Science of Skin'. The meeting included updates in systemic and biologic therapies for psoriasis and new developments in the treatment of skin cancer, as well as hot topics such as onychomycosis and hair loss. In this first session, Prof Thaçi and Prof

Augustin reviewed advances in the systemic treatment of psoriasis and explored how successful development of new treatments has led to an improved understanding of underlying disease processes. With a particular focus on the history of treatment with fumaric acid esters (FAE), the speakers explored the impact of the introduction of dimethylfumarate (DMF) monotherapy on knowledge of psoriasis and its treatment. Other topics included the complexities of treatment selection, the importance of meeting patients' expectations, and the significant role that biomarkers and personalised medicine will have in future treatment decisions.

Treating Psoriasis

Professor Diamant Thaçi and Professor Matthias Augustin

Treatment History

Psoriasis has been treated by physicians for thousands of years, with evidence suggesting that the use of topical treatments and sunlight therapy dates back as far as the ancient Egyptians and Greeks.[1] Despite this long history, the first effective treatments were not identified until the early 20th century. The development of ultraviolet (UV) phototherapy for skin diseases won a Nobel Prize in 1903, with its use in psoriasis first described in 1925; however, it was not regularly used as a treatment until the 1950s.[1,2] The prevalence of syphilis during this time meant that dermatologists often also specialised in venerology, and with more patients dying of syphilis than cancer, new treatments were urgently sought. A breakthrough came in 1917 with the identification of arsphenamine, an arsenic-based compound that became the first parentally administered treatment in dermatology and venerology.[3] Dermatology has since become a distinct specialism and huge advances have been made in understanding and treating dermatological conditions, including psoriasis.

Today, psoriasis is thought to affect between 0.5% and 11.4% of the population;[4] however, a wide variation in presentation complicates treatment identification and selection. Differences in severity and location of skin symptoms, and the presence of a variety of related comorbid conditions, means that treatments are not equally effective for all patients. Although environmental factors are involved in the pathogenesis of psoriasis, particularly in older patients, research has revealed an important genetic component. Among cases occurring before the age of 20 years, approximately 50% have a genetic cause, and an abundance of genes are known to be involved, with 16 new loci identified in recent years.[5] Most of the identified genes are involved in the differentiation and regulation of lymphocytes, but others are responsible for responses to stimuli, regulation of adaptive immune responses, and other signalling pathways.[5] These discoveries have linked psoriasis with other inflammatory diseases, including inflammatory bowel disease and arthritis, and revealed that psoriasis is much more complicated than originally thought.[6] In the past 20 years, the introduction of new systemic and biologic medicines has changed the treatment landscape for psoriasis and further improved the understanding of underlying disease processes (Figure 1).[7] Treatment with tumour necrosis factor (TNF) inhibitors led to psoriasis being considered as a systemic disorder and broadened understanding of the disease processes.[8,9] Today, a wide range of treatment options are available to patients presenting with mild-to-severe disease, including topical therapies, phototherapy, and systemic and biologic medicines.

Treatment Selection and Guidelines: Meeting Patient Expectations

The increase in the availability of effective therapies in recent years has improved the outlook for patients, but it poses a challenge for dermatologists in selecting the most appropriate treatment for each patient and has resulted in a need for frequent updates to treatment guidelines. Analysis of the German PsoBest registry, the main registry of systemic treatments for psoriasis, found that during 2015–2016, the most frequently prescribed systemic treatments were FAE, followed by methotrexate and then biologics (PsoBest registry data, 4/2018).

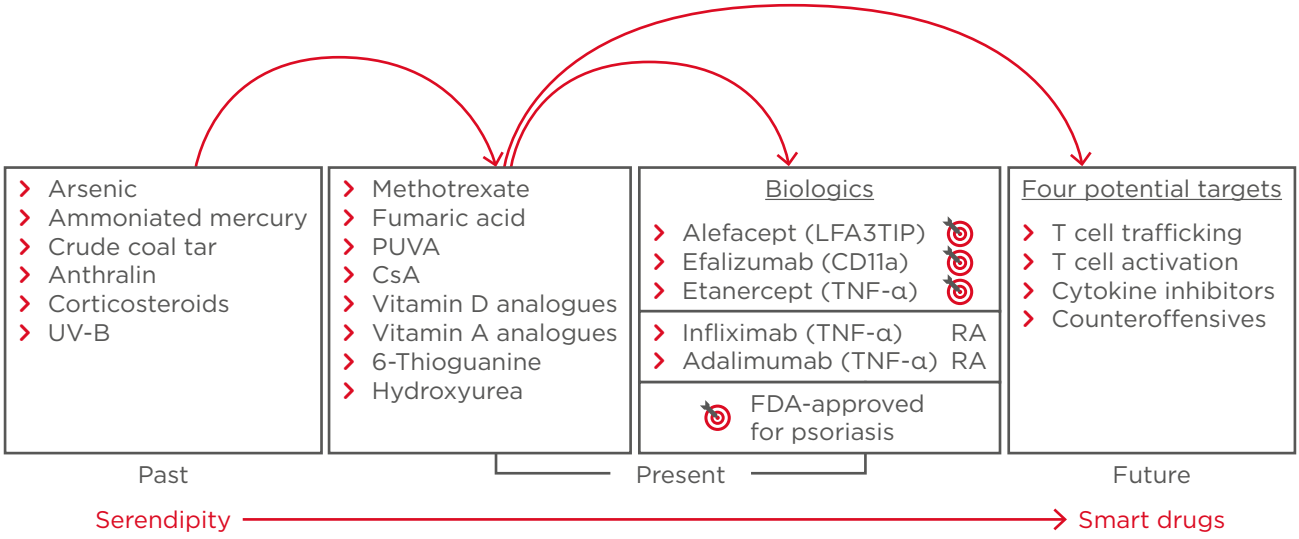


Figure 1: Developments in psoriasis treatment, from arsenic to biologics.

CsA: cyclosporin A; FDA: U.S. Food and Drug Administration; PUVA: photochemotherapy; RA: rheumatoid arthritis; TNF: tumour necrosis factor; UV-B: ultraviolet-B.

Adapted from Nickoloff and Nestle.[7]

The advent of more effective treatments has led to a change in patients' and physicians' expectations of treatment outcomes. As a result, drug developers are now bound by more stringent measures of efficacy than in the past, with corresponding updates to treatment guidelines. For patients with severe psoriasis, treatment goals in 2004 were for a 50% improvement in Psoriasis Area Severity Index (PASI) 50.[10] The introduction of TNF inhibitors opened up the potential for PASI 75 as a realistic treatment target for patients with severe disease.[8,9] More recently, development of anti-interleukin (IL)-17 agents has changed the field of expectation for severe psoriasis once again, with PASI 90 achieved by >70% of patients and PASI 100 achieved by more than one-third of patients.[11,12] The better response rates observed in these clinical trials could translate to revisions of treatment guidelines to include >90% improvement in PASI score as a realistic treatment goal for patients with severe psoriasis.

The most important issues for patients, in terms of treatment outcomes, are fast and sustainable improvements in skin symptoms without the fear of a subsequent relapse.[4] As discussed, systemic and biologic medicines have transformed the treatment landscape for patients with severe disease, while patients with mild psoriasis often have excellent responses to topical treatments or phototherapy. However, patients with moderate disease often fall somewhere in between, with symptoms that are too severe to respond to topical treatment or phototherapy but too mild to be treated with systemic agents. In these cases, treatment choice may be guided by the presence of comorbidities.

Approximately 30% of patients presenting with psoriasis develop psoriatic arthritis.[13] For a patient with moderate skin symptoms displaying evidence of arthritis, it is essential to initiate early intervention with systemic treatments to prevent radiographic progression of arthritis, which can result in irreversible joint damage.[13] Treatment goals for patients with psoriatic arthritis are focussed on improvements in tender and swollen joints and enthesitis, alongside improvements in skin symptoms (PASI score and body surface area) and quality of life.[14] Conventional TNF inhibitor treatments only achieve <60% response rates for American College of Rheumatology (ACR) 20% improvement criteria (ACR20) in patients with psoriatic arthritis, leaving much room for improvement.[15-18] More recently, phosphodiesterase-4 and IL-17 inhibitors have shown more promising results.[19] Recent guideline updates differentiate psoriatic arthritis

from psoriasis for the selection of biologic treatments.[20,21] Future guideline updates must consider optimal treatments for disease severity and comorbidities, as well as patients' expectations for treatment outcomes.

Understanding Disease Processes

A broader understanding and better treatment of systemic inflammation are improving outcomes for a wide range of diseases with an inflammatory component, including psoriasis.[22,23] For example, the presence of low-grade systemic inflammation in psoriasis, involving a variety of cytokines, may explain why phototherapy focussed solely on the skin is not effective in some cases. In addition, low-grade chronic systemic inflammation is thought to be responsible for a range of diseases, including nonalcoholic fatty liver disease, psoriasis, and cardiovascular disease.[24] Thus, while patients with psoriasis have an increased risk of developing cardiovascular disease, adequate treatment and disease control can reduce this risk, a finding which has led to psoriasis being used as a model to explain inflammatory artherogenesis.[25] Similarly, research into psoriasis and psoriatic arthritis has identified the fundamental similarity of these diseases at the molecular level. Variation in the levels and location of expression of cytokines in the IL-17/IL-23 pathway is associated with a wide range of pathological processes, including enthesitis and bone formation, differentiation of osteoclasts (leading to bone erosion), proliferation of fibroblasts (leading to matrix destruction), and proliferation of keratinocytes (leading to skin inflammation and plaque formation).[26] Data from patients enrolled in psoriasis registries have also shown improvements in other comorbidities, such as depression, following systemic treatment for skin symptoms.[27]

Systemic Psoriasis Treatments: Fumaric Acid Esters and Dimethylfumarate

While much attention is given to new biologic treatments, advances in other systemic therapies have also improved treatment options and outcomes for patients with psoriasis. FAE have been used to treat psoriasis for almost 60 years,[28] and they also have diverse applications beyond psoriasis, both within biomedicine and even in the food, agriculture, and green chemistry industries.[29] Historically, it was thought that psoriasis was caused by a fumaric acid deficiency that could be cured by conversion of FAE to fumaric acid in the body. While FAE are indeed converted to fumaric acid, it is now known that fumaric acid deficiency is not the cause of psoriasis but that multiple inflammatory pathways are responsible.[5,6]

All FAE treatments contain DMF as the main active component.[30,31] When administered orally, as with most psoriasis treatments, DMF is primarily metabolised via the citric acid cycle and eliminated as CO_2 during exhalation.[30] One of the stages of this process, occurring following absorption from the gut, is the partial conversion to monomethylfumarate (MMF), which, unlike DMF, can be detected in the circulating blood. It is believed that DMF and MMF act at different body sites, including the mucosa, skin, and the central nervous system.[30] Although further research is needed to uncover the full effects of DMF and MMF, they are known to have multimodal activity, influencing multiple cytokine and lymphocyte pathways, including glutathione modulation and signalling via nuclear factor erythroid 2-related factor (Nrf2), nuclear factor kappa B (NFκB), and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway.[30,32-34] This activity results in broader effects in the body beyond skin symptoms; FAE have been shown to affect endothelial and cardiovascular functions,[35-37] restore apoptosis sensitivity, and reduce melanoma growth rate and progression to metastases.[38,39]

Since their introduction in the 1950s, FAE have been and remain widely prescribed.[40,41] In psoriasis, FAE act relatively slowly but week-by-week improvements in skin symptoms are usually noted. Combining FAE with topical treatment or phototherapy can result in more rapid improvements and allow for dose reduction compared to FAE treatment alone.[42,43] The speakers highlighted that FAE are a suitable treatment option for a large proportion of patients since they tend to have similar efficacy regardless of the location of psoriasis; notable exceptions are nail psoriasis or psoriatic arthritis, which do not respond as well to FAE treatment. German treatment guidelines indicate FAE as a first-line systemic treatment prior to biologic therapies for all but the most severe psoriasis cases, and for those with evidence of arthritis.[20] A discussion of dosing

strategies indicated that while there is no severity threshold for treatment with FAE and dosing strategies can be flexible to meet patient needs, faster-acting treatments should be considered for patients with severe disease. When it is appropriate to use FAE, dosing usually starts low, increasing gradually until a response is achieved or adverse events trigger a dose reduction. Treatment can then continue providing it remains safe and effective, and dosing can be gradually increased, decreased, or stopped and restarted according to response. Following a response, the dosage can be reduced every 4 weeks by reducing the number of tablets taken daily by one.

The importance of discussing potential side effects with patients when considering FAE therapy was also raised, since this represents the best route to ensuring that patients adhere to treatment. Gastrointestinal complaints and flushing can be common and of particular concern to patients; however, they are often temporary and mild in severity. Patients who develop side effects might temporarily reduce their daily dose. If this approach is unsuccessful, it may be necessary to discontinue treatment.

Lymphopenia is a particularly important adverse event associated with FAE treatment, since prolonged lymphocytopenia is associated with the development of progressive multifocal leukoencephalopathy, a rare adverse event.[44] Identification of lymphopenia and careful monitoring and management is important to minimise the risk of developing progressive multifocal leukoencephalopathy.[45]

Lessons from Real-World Registry Data

Efficacy, safety, and treatment patterns with FAE therapies have also been assessed in real-world studies, including patient registries. The German PsoBest Registry includes 7,600 patients with psoriasis under the care of >850 dermatologists.[46] The registry employs robust methods, with the researchers analysing treatment patterns, efficacy, and safety for patients every 3 months from the first systemic treatment dose.[46] A total of 10 years of follow-up data are now available for the first patients enrolled. Analyses of the data have suggested that patients treated with FAE have a greater need for improvement in skin symptoms and lesser need for improvement in pain than patients receiving biologics or alternative systemic therapies.[47]

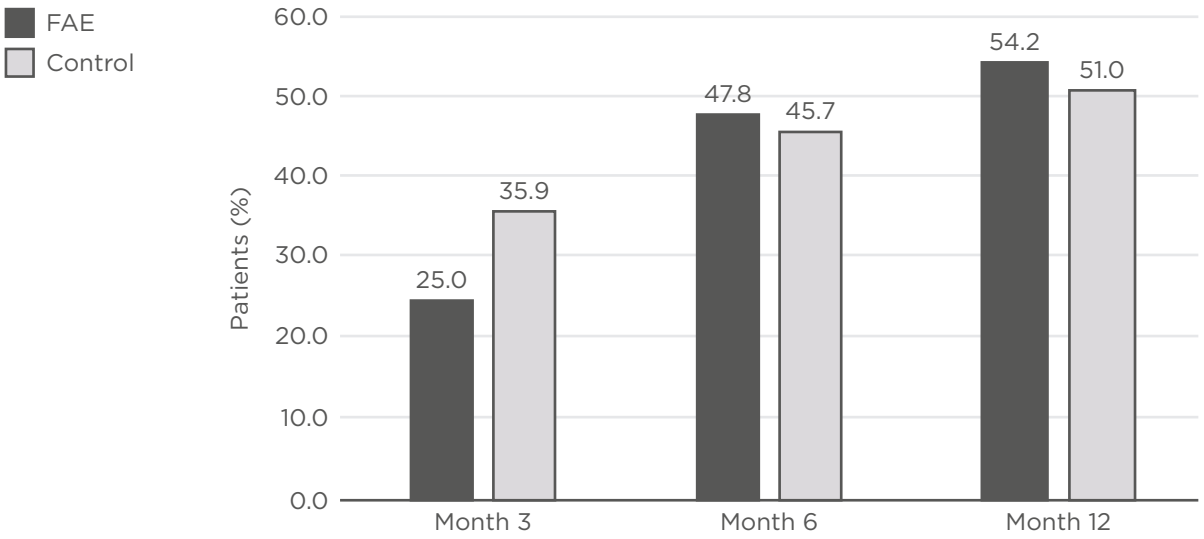
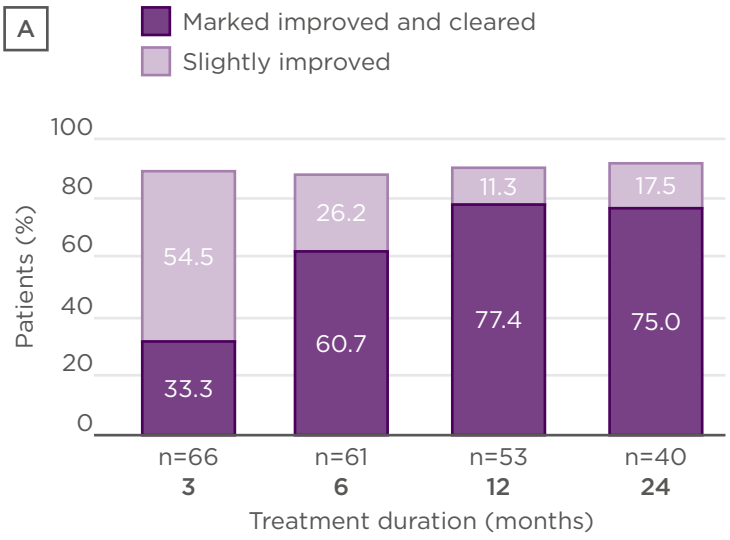


Figure 2: Proportion of patients achieving Psoriasis Area Index Severity 75 at Month 3, 6, and 12 after enrolment to the PsoBest registry and treatment with the first systemic agent.

A comparison was made between FAE and other nonbiologic systemic agents (control). The control group represents all available systemics prescribed to enrolled patients, most commonly methotrexate (PsoBest registry data, 4/2018).

FAE: fumaric acid esters.

A



B

Most frequently stated comorbidity	n	%
Metabolic and nutritional disorders	44	63.8
› Diabetes	19	27.5
› Hyperlipidaemia	15	21.7
› Hypercholestrolaemia	13	18.8
› Obesity	7	10.1
› Hyperuricaemia	4	5.8
Vascular diseases	44	63.8
› Hypertension	42	61.1
› Varicosities	3	4.3
Cardiac diseases	14	20.3
› Coronary heart disease, angina pectoris, ischaemic heart disease	6	8.6
› Myocardial infarction	4	5.8
› Cardiac insufficiency	2	2.9

Figure 3: A) Improvement in skin symptoms among patients comedicated with fumaric acid esters and agents to treat comorbidities, according to the Physician's Global Assessment. In the FUTURE study, 67% of patients at 6 months and 78% at 24 months who received FAE had a response of markedly improved or clear (21% of patients comedicated);[41] B) The most common comorbidities in the FACTS study.

Adapted from Thaçi et al.[50]

This could be explained by the lower prevalence of arthritis among these patients. Efficacy comparisons with other agents based on achieving PASI 75 have suggested that although they have slower onset of efficacy, FAE have similar or greater efficacy compared with nonbiologic systemic therapies, the most common of which being methotrexate, over the longer term (Figure 2).

Safety analyses using PsoBest data indicated that FAE were associated with higher rates of skin reactions (erythema and flushing), lymphopenia, and gastrointestinal disorders (e.g., cramps, diarrhoea), and lower rates of infections and infestations, when compared with other nonbiologic systemic therapies (PsoBest registry data, 4/2018). However, these adverse events were generally considered to be manageable and there were no significant differences in serious events in any system organ class between FAE and other systemic therapies.[48] Similar event profiles were reported for FAE and DMF during the Bridge study,[49] although this study compared both FAE and DMF with placebo, rather than with other nonbiologic systemic drugs. Drug survival for FAE is good, with 60% of patients remaining on treatment after 2 years. This is comparable to the drug survival observed with other nonbiologic systemic agents but is lower than the drug survival levels observed with the biologic agents adalimumab and ustekinumab. (PsoBest registry data, 4/2018).

The Effects of Drug–Drug Interactions and Coexisting Medical Conditions in Treating Psoriasis

The underlying systemic inflammation associated with psoriasis means that patients are likely to present with at least one medical condition for which they are receiving other medications. The most common comorbidities include metabolic and nutrition disorders (e.g., diabetes and hyperlipidaemia) and cardiac disease.[50] Due to this, drug–drug interactions are an important consideration when determining optimal treatment for psoriasis, and methotrexate, for example, is known to interact with other drugs.[51] FAE are not metabolised via common pathways, e.g., via cytochrome 450, and coadministration with medications used to treat the most common comorbidities does not appear to have a large impact on their efficacy (Figure 3).[41,50] In addition, patients receiving

FAE are able to mount an effective immune response to vaccinations during therapy.[52] Reported changes in laboratory parameters are often insignificant, rarely requiring dose modification of FAE.[50] However, patients with psoriasis should be screened for medical conditions, including pregnancy, prior to starting medication to ensure they are receiving the optimum treatment strategy.

Future Perspectives

While the active component of FAE treatment is DMF, many agents include additional FAE in combination with DMF. Recent research has focussed on isolating DMF for use as a monotherapy and clinical trials have demonstrated similar efficacy and safety of FAE combination and DMF monotherapy,[49] as well as no negative effects on immunisation or seroprotection.[52] In June 2017, Skilarence® (Almirall, Barcelona, Spain), a DMF monotherapy, became the first FAE to be licensed by the European Medicines Agency (EMA) for the treatment of moderate-to-severe plaque psoriasis in adults in need of systemic medicinal therapy.[53,54] Prior to this approval, Fumaderm® (Biogen Idec GmbH, Ismaning, Germany), a combination of four FAE, was the only FAE treatment approved in Europe; however, Fumaderm was only licensed for use as a psoriasis treatment in Germany. DMF monotherapy has been available in Germany since October 2017, during which time data from the PsoBest registry have shown that most patients receiving Fumaderm have since been switched to receive DMF monotherapy (PsoBest registry data, 4/2018).

Many new biologic treatments have recently been approved for the treatment of psoriasis and/or psoriatic arthritis, and several more are in development. To date, biologic medicine development has focussed on the IL-17/IL-23 pathway, for which promising results have been shown in both diseases.[11,12] Future research is likely to focus on maintenance of remission after treatment withdrawal. Recent trial data suggest that long-term remission (PASI 75 and PASI 90) could be possible following anti-IL-23 treatment, even when treatment is stopped.[55,56] Future improvements in treatment availability and selection will rely on the identification and monitoring of biomarkers, leading towards personalised medicine and precision healthcare, ensuring patients' individual needs are met.

Conclusion

Understanding of the complexities of psoriasis has greatly improved over the past 20 years, coinciding with improvements in disease control and management. The advent of systemic and biologic therapies has revolutionised treatment for patients but has also led to higher expectations for treatment outcomes. The drug development pipeline for psoriasis is very promising and dermatologists should expect ongoing changes to treatment guidelines in response to future drug approvals. Since their introduction >50 years ago, FAE have become a valuable and cost-effective systemic treatment choice for moderate-to-severe psoriasis. FAE are suitable for the short and long-term treatment of patients receiving comedication, provided that the dose is individually tailored to the patient's needs regarding severity of disease and comorbidities. As such, FAE are now recommended as a first-line treatment option in new German treatment guidelines.[20] Recent advances have led to the European approval of Skilarence, a DMF with similar efficacy and safety to FAE, which has increased patient access to this important treatment. Future developments in psoriasis treatment will focus on long-term maintenance of response and identification of biomarkers for a personalised approach to treatment.

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Anti-Tumour Necrosis Factor in Inflammatory Bowel Disease: Inventory and Outlook

This symposium took place on 15th February 2018, as part of the 13th European Crohn's and Colitis Organisation (ECCO) Congress in Vienna, Austria

Chair People: Gert Van Assche[1]

Speakers: Raja Atreya,[2] Yoram Bouhnik,[3] Fraser Cummings,[4,5] Geert D'Haens[6]

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Meeting Summary

Biosimilars have contributed substantially to the evolving therapeutic landscape for inflammatory bowel disease (IBD), enabling healthcare systems to offer access to a broader range of therapies at an affordable price. Despite the increasing confidence healthcare practitioners (HCP) place in the safety and efficacy of biosimilars, uncertainty remains around best practice when switching a patient from a reference product to a biosimilar. This symposium aimed to uncover the importance of a managed switch programme by exploring real-world data from the University Hospital Southampton NHS Foundation Trust, Southampton, UK, and University Hospital Erlangen, Erlangen, Germany. The roles of therapeutic drug monitoring and predictive outcome scoring were discussed based on evidence from the SECURE and the CREOLE studies, respectively. Finally, in light of the recent European Union (EU) approval of adalimumab biosimilars, the future therapeutic landscape and how physicians and patients can make well-informed decisions when multiple versions of the same biologic are available were discussed.

Introduction

Prof Van Assche moderated a highly interactive podium discussion between the faculty members and the audience, addressing key clinical points around switching patient treatment to a biosimilar. Interactive technology was used to maintain continuous dialogue with the audience.

Since the introduction of infliximab almost two decades ago, anti-tumour necrosis factor (TNF) biologics have revolutionised treatment for IBD. Several other anti-TNF biologics have followed, in addition to two alternative agents with novel mechanisms of action: vedolizumab, an integrin antagonist that targets the gut-specific α4β7 integrin for inhibition,[1] and ustekinumab, which targets the upstream regulatory cytokines interleukin-12 and interleukin-23 to disrupt the inflammatory cascade.[2] Recent approvals of biosimilars have also widened treatment options by providing cost-effective alternatives to reference products. To date, there are >20 biosimilar products licensed in Europe, including biosimilars for infliximab (e.g., CT-P13 and SB2) and etanercept (e.g., SB4 and GP2015). According to Prof Van Assche: "Anti-TNF will be a mainstay treatment for the next 10 years for IBD. Additionally, with adalimumab biosimilars and oral therapeutics on the horizon, gastroenterologists will be empowered with exceptional choices when selecting the best treatment for their patients with IBD." With several years of real-world experience now supporting confident use of biosimilars, the practicalities of introducing biosimilars effectively into clinical practice have come to the fore; indeed, when the audience was asked whether they already used biosimilars in their daily clinical practice, half of the attendees reported that they were, with approximately 60% of the remaining half confirming they were happy to switch their patients to a biosimilar.

Best Practices and Key Considerations for Switching: Lessons from Case Studies

A managed switch programme using a gainshare model can deliver significant cost savings and investment in clinical services while maintaining comparable clinical responses, patient-reported outcomes (PRO), and drug persistence. As Dr Cummings explained: "In a gainshare model, you make an investment or a change in your practice and the benefits are shared between the different stakeholders involved in the process, therefore including the payers, the hospital, the clinical team, and the patients." Dr Fraser Cummings, Prof Raja Atreya, and Prof Geert

D'Haens shared their personal experience, discussing the key elements that made their programmes successful (Figure 1).

At Southampton General Hospital, Southampton, UK, Dr Cummings and his team switched 143 IBD patients from originator infliximab to CT-P13.[3] Dr Cummings summarised: "We saw no change in immunogenicity or in objective inflammatory markers; in general, about 20% of patients on infliximab stop treatment within a calendar year, so it was important that we demonstrated no difference in the drug persistence rate after we switched patients. We showed a very clear decrease in drug acquisition costs and astatistically significant improvement in patients' reported quality of life. The latter could beattributed to the additional resources that we acquired as a direct result of the cost savings."

Similarly, Prof Atreya participated in the implementation of a gainshare model in his department at University Hospital Erlangen, Erlangen, Germany, where 119 patients with ulcerative colitis (UC) or Crohn's disease (CD) were successfully switched from reference infliximab to SB2 between February and April 2017.[4] Prof Atreya reported: "6 months after switching, most patients remained in clinical remission, having a Mayo score of 0 or 1 for patients with UC, and Harvey–Bradshaw index <5 for patients with CD, with no statistical significance in the changes in clinical outcomes compared to Week 0. Median trough levels measured through therapeutic drug modelling (TDM) revealed no statistically significant change from baseline to Week 24."

Likewise, Prof D'Haens and his team successfully switched 100 patients with CD from reference infliximab to biosimilar CT-P13 in the SECURE study,[5] which assessed serum drug concentration and clinical activity. "There was no difference in the serum concentration of CT-P13 at Weeks 8 and 16 following the switch compared with reference infliximab. In terms of immunogenicity, the signals were very reassuring: C-reactive protein values remained close to zero, despite a slight variation, which is to be expected. These favourable outcomes prompted switching of 95% of patients at the institute to a biosimilar."

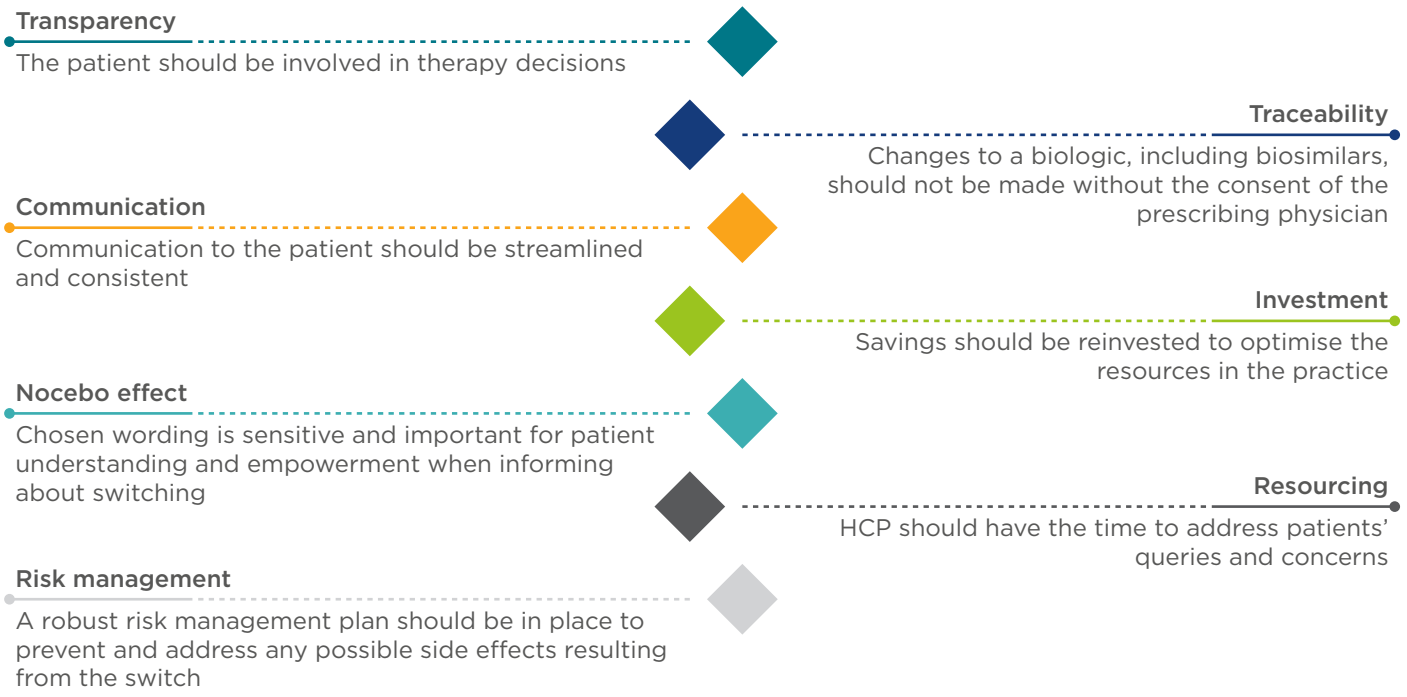

Figure 1: Best practices to create a reference biologic-to-biosimilar switching model.

HCP: healthcare practitioner.

Key Aspects of Patient Communication

When discussing effective ways to involve patients in shared decision-making regarding switching, Dr Cummings reported: "At a very early stage we brought in a patient panel, which is a group of people that meet with us every month or couple of months to discuss the service and the care that we are delivering. They are also very useful for discussing ideas and research proposals. We spent a couple of hours discussing a potential biosimilar switch with our patient panel and this was key to the successful outcomes of our switching process. Patients were thus able to make informed decisions and become an integral part of the switching model. Physicians used this time to develop new ideas to improve their research, delivery of care and the process they were putting in place, to effectively develop the switching programme."

During the programme, Dr Cummings identified that: "Infusion nurses as well as specialist nurses play a critical role as information providers. Patients have the most concerns at the time preceding and during the switch to a biosimilar, and often the infusion nurses are the HCP that spend some time addressing patients' feelings and concerns." Infusion nurses were, therefore, educated about biosimilars and allowed to have a space where their potential concerns about switching could be addressed, in order for them to both understand and be confident in the use of biosimilars, and also transfer this confidence to the patients. Dr Cummings explained: "It is important that the nurses are not anxious about switching, as HCP communicate with the patients both verbally and with body language."

"Another key source of information are physicians. At the departmental meeting, physicians were able to address any queries surrounding reference originator-to-biosimilar switching. Communication with patients was thus streamlined to ensure patients received the same message from all parties. Additionally, HCP were reassured by a robust risk management plan so that if there was a problem in switching patients, they would find it quickly and be able to react appropriately. This gave HCP the confidence to engage in the plan and feel reassured about their patients' care," Dr Cummings stated.

During the same programme, as additional support, patients were given a letter to explain what a biosimilar is. Dr Cummings explained that: "Patients were able to discuss the contents of the letter with the specialist nurses and had time to consider its contents at home. Patients then confirmed whether they were happy to go ahead with the switch at their next infusion appointment."

Addressing the Nocebo Effect

Physician-to-patient communication is also key to reducing the likelihood of a nocebo effect. The nocebo effect is the negative equivalent of the placebo effect, and has historically been shown to have a detrimental impact on treatment adherence and outcome in several diseases.[6] Negative perceptions due to lack of understanding and knowledge about biosimilars can result in a nocebo effect;[7] therefore, minimising the nocebo effect by increasing knowledge and understanding about biosimilars is crucial when switching patients from a reference biologic to a biosimilar. "Patients who are in remission following a very severe disease and receiving a reference biologic are particularly reluctant to change to a biosimilar. Physicians are aware that with all TNF inhibitors there will be a loss of treatment response over time, but for patients, once they switch, they assume that the loss of response is due to the biosimilar," Prof Yoram Bouhnik stated.

Dr Cummings suggested a way to overcome this: "Prior to the switch, discuss the side-effects patients might already be experiencing and potential flares on the reference drug with patients; this provides an opportunity to reduce possible nocebo effects following the switch to biosimilar." According to Dr Cummings, the implementation of this element in his switch programme was associated with the lack of changes in side effects reported when patients were switched to the biosimilar.

Reinvesting Savings into the Clinic

If the nocebo effect is minimised, uptake and adherence to biosimilars can be improved and ultimately result in savings for departments and healthcare systems. These savings can then be realised within the department if a gainshare model is used; i.e., an agreement that some or all of the savings made are retained within the department initiating the change, as Dr Cummings clarified. He added: "This scheme worked because all the key participants benefited from the model; in our case, this included first and foremost the patients, but also the institutions, the pharmacy, the payers, and the faculty."

"A key element for the success of a gainshare model is the clear incentivisation to the clinic providing the services so that these are implemented and acknowledged," stated Dr Cummings. In his case, the switch to biosimilars led to a reduction in drug acquisition costs of £40,000–60,000 per month. "My team was highly incentivised, as the savings were used to acquire an additional IBD nurse, as well as a secretary to support both the physicians and the nurses. This definitely contributed to making the model a success." Dr Cummings believes: "It is always important to acknowledge the amount of work involved when switching patients… …but the incentivisation depends on the healthcare system. Switching to biosimilars takes time, energy, and resources, and that is part of the reason why we made sure to get our share reinvested in our service. The significant increase in patient outcome measures noted in the programme could be attributed directly to the cost savings with investment in more resources for patients. To this point, it is crucial to notice how the uptake of biosimilars has been much lower in models where the share was not reinvested in the institution."

Similarly, Prof Atreya believes the success of the switch at University Hospital Erlangen can be attributed to the use of a gainshare model, the financial benefit of which enabled his clinic to hire a second physician in the IBD unit: "As switching is an emotional process for patients, the availability of a second physician enabled us to spend more time with patients and introduce them comprehensively to the topic of switching in a much more relaxed manner. This was particularly important for the patients who have been in remission on the reference biologic for many years. Therefore, the gainshare model benefited both patients and HCP within the department." Prof Atreya has had experienced patients querying whether the switch in treatment is solely to benefit the hospital financially: "Patients can wrongly perceive the switch to a less expensive product as a switch to a less effective and potentially more harmful medication. It is important to highlight to the patient how this switch in treatment leads to the financial benefits to the hospital that are then utilised within the department, such as the addition of new staff, more resources to support patients through the switch process, and shorten waiting times for clinical visits. This allows the patient to experience the benefits directly." As a result of the success of his model, Prof Atreya confirmed that no patient had left the institute to seek care at another institute, and concluded: "Taking the time to explain to patients and paying attention to their worries is of outmost importance."

Results from a symposium polling question revealed that the biggest obstacle in implementing a gainshare model within an institute was that the cost savings could not be retained by the department. To ensure that the savings would be invested into the department and not in the hospital cost improvement programme that is in place in the UK, Dr Cummings established very strong relationships with the payers and the hospital management at a very early stage. He argued: "The financial success can only come true if the investors understand that using this gainshare model to develop a biologic service will be both cost effective and cost efficient, and will provide the patients with higher-quality care. The gainshare model should, therefore, be pitched as a model that can save you money and provide better care."

Switching Between Biosimilars

The implementation of a successful reference biologic-to-biosimilar switch model can result in significant cost savings for the department. However, with an ever-widening IBD treatment

armamentarium, clinics need to also start considering best practices surrounding a biosimilar-to-biosimilar switch. When asked, the audience revealed mixed results relating to their confidence in terms of a biosimilar-to-biosimilar switch. Prof D'Haens shared that he had previously performed a second switch; however, he explained: "Both biosimilars were manufactured in the same factory, and hence were inherently the same; had these been produced in a different environment, I would have been unsure."

According to Prof Atreya, "if we see no 'red flags' on the first switch, why should we expect any surprises on the second?" In agreement, Dr Cummings clarified: "Intellectually, switching to another biosimilar should be no different than switching from a reference biologic to another biosimilar. It is important to acknowledge, however, that regulatory bodies, such as the European Medicines Agency (EMA), usually compare the reference biologic to the biosimilar and not the biosimilar to another biosimilar. Recent data suggest that there are no differences between biosimilars at the characterisation level. Additional data have demonstrated cross-reactivity between SB2, CT-P13, and between both of them and the reference biologic;[8] we can therefore assume that the results could be extrapolated into clinical practice and that there would not be a problem. Data coming out in the coming year will hopefully confirm this theory and unveil the feasibility of a biosimilar-to-biosimilar switch, or potentially a biosimilar-to-reference biologic switch, should the cost permit it."

Using Therapeutic Drug Monitoring

Regardless of the type of switching, therapeutic drug monitoring (TDM) can be a helpful tool to monitor patients. In a symposium poll, 53.2% of the audience responded that they use TDM to optimise biologic effectiveness in specific patients, while 19.1% replied that they use it regularly.

During the SECURE study, Prof D'Haens used TDM to monitor the switch from infliximab to CT-P13: "TDM was introduced in our SECURE study to compare serum concentrations, presence of antidrug antibodies and C-reactive protein levels between reference biologic and biosimilar, and to provide patients with additional support when considering switching. The data gave the patients further confidence that switching to a biosimilar is a safe and viable approach." Prof D'Haens commented: "We used TDM extensively and intensively during the first year of the study but have used it less as our confidence in switching increased."

Predictive Factors: Future of Treatment Success?

Predictive factors that could forecast the success of biologic therapy (regardless of whether a reference product or biosimilar) in biologic-naïve patients could significantly impact the patient's quality of life, allowing the patient to receive the most appropriate treatment sooner. Patients with CD and stricture, the most common complication in CD patients, were initially contraindicated for anti-TNF treatment due to the increased risk of intestinal obstruction. Treatment of this condition is difficult, and these patients are often recommended for surgery. However, evidence from the TREAT and the ACCENT registries[9] suggests that infliximab treatment is not associated with an increased risk of obstruction in patients with or without intestinal strictures at baseline. Prof Bouhnik therefore initiated the CREOLE study[10] to establish the effectiveness and safety of adalimumab in patients with CD and symptomatic small bowel stricture.

In this study, 97 patients were included and received treatment with adalimumab. The primary endpoint, defined as adalimumab success at Week 24, was observed in 62 of 97 patients. Moreover, multivariate analysis found seven clinical and imaging parameters that were independently associated with the success of adalimumab treatment, and could therefore be used as predictive markers (Figure 2). To calculate the CREOLE score, one point should be given to each factor: patients with a score >3 will most likely succeed under adalimumab treatment (>90%), while patients with a score <2 (<10%) should instead be treated with surgery.

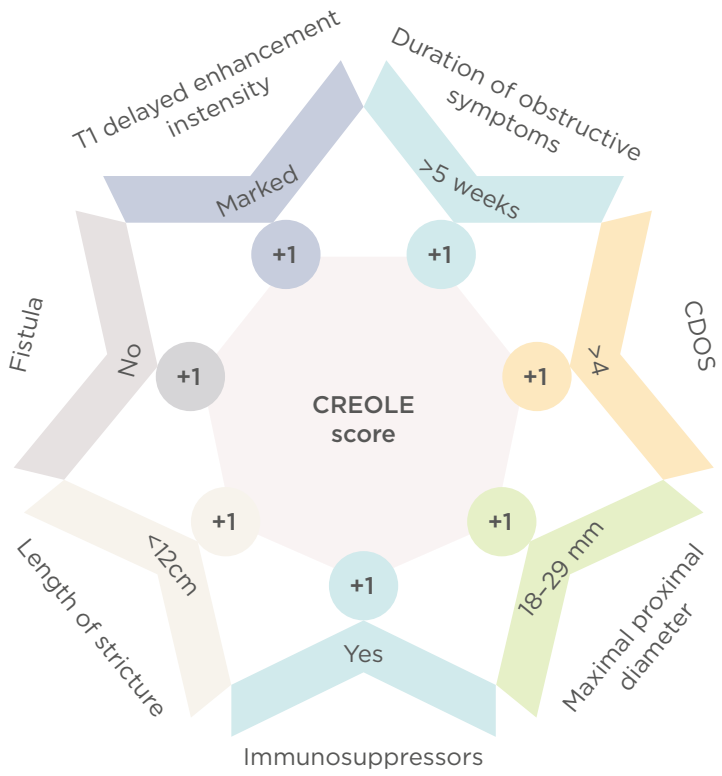

Figure 2: Predictive markers to calculate the CREOLE score.

CDOS: Crohn's Disease Obstructive Score.

Prof Bouhnik reported: "The use of the CREOLE score as a prognostic factor was associated with >50% of patients initially treated with adalimumab being surgery-free after a median follow-up of 4 years; however, an independent study is required to validate these innovative results."

Biosimilars in the Future

"The upcoming adalimumab biosimilars, which will be available as a subcutaneous injection, as opposed to an intravenous infusion, will have a significant impact in the field of biosimilars," predicted Prof Atreya. "However, in terms of safety and efficacy, the new biosimilars will probably perform as well as their reference biologic, in the same way that infliximab biosimilars had performed as well as infliximab; all biosimilars have to fulfil a very diligent comparability exercise and clinical tests before entering the market. The validity of these tests has been shown by infliximab biosimilars; therefore, if the new biosimilars are able to pass their preclinical tests, then we can expect them to be functional clinically." A good example is the biosimilar SB5. "The data[11,12] are very reassuring, as they reveal no clinically meaningful differences in terms of pharmacokinetics, safety, and efficacy," Prof D'Haens stated.

Conclusion

Biosimilars have been shown to have similar critical quality attributes, such as physicochemical qualities and Fab/Fc-related biological activity, equivalent pharmacodynamics and efficacy, and similar safety profiles to their reference biologic. Accumulated evidence from real-world experience with biosimilars has confirmed biosimilarity, leading to an increase in physician confidence in their understanding and use. However, further information on how to implement biosimilars into their clinic efficiently and effectively is required.

The case studies discussed during this meeting highlight that the implementation of a switching programme can offer substantial benefits to both the institute and the patients. Prof D'Haens concluded: "The three key factors that make a switching programme both a financial and a clinical success are transparency: always tell the patients what you are doing, and what you know; traceability: always know what the patient received and when; communication: ensure that everyone is telling the same story." With careful planning, financial benefit can be reinvested in the practice to result in additional resources and support, which will directly benefit all stakeholders.

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Pyruvate Kinase and Gastric Cancer: A Potential Marker

EDITOR'S PICK

My Editor's Pick for this edition is a captivating review by Macedo et al. on a rather new topic, which concludes that PKM2 and PDK1 measured in the blood or stools of patients, when analysed in combination with CA72-4, are good markers for gastric cancer and are predictors of poor survival. This combination of two biomarkers could help in monitoring the response to treatment and detecting progression or relapse of gastric cancer. This paper opens new avenues for research into novel drugs targeting PKM2.

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Abstract

Gastric cancer is the second most common cause of cancer-related deaths worldwide, and the 5-year overall survival rate for advanced gastric cancer is ≤25%. Metabolism is a critical process for maintaining growth and other functions in cancer cells; in these cells, the metabolic process shifts from oxidative phosphorylation to aerobic glycolysis and the expression of pyruvate kinase (PK) splice isoform M2 (PKM2) is upregulated. A PubMed search focussing on PK in gastric cancer was conducted and 32 articles were initially collected; 12 articles were subsequently excluded from this review. PKM2 is responsible for tumour growth and invasion and correlates with short survival times and cancer differentiation. Pyruvate dehydrogenase kinase 1 is associated with cell proliferation, lymph node metastasis, and invasion. Measurement of PKM2 or pyruvate dehydrogenase kinase 1 in

the blood or stools could be a good marker for gastric cancer in combination with the glycoprotein CA72-4. The review arose from the need for new biomarkers in the management of gastric cancer and had the primary objective of determining whether PK could be used as a marker to diagnose and monitor gastric cancer.

INTRODUCTION

Gastric cancer is the fourth most common cancer in men worldwide and the second most common cause of cancer-related deaths worldwide.[1,2] In 2008, approximately 700,000 deaths were related to gastric cancer, equating to 10% of all cancer-related deaths, and 989,600 new cases of gastric cancer were diagnosed, representing 8% of all new cancer cases.[1] The number of gastric cancer patients diagnosed in the early stage of the condition is increasing due to improvements in early diagnosis; however, many cases are still found in the advanced stage.[1,2] For advanced gastric cancer, the 5-year overall survival rate is ≤25%.[3] Therefore, identification of molecular therapeutic targets and novel biomarkers for early diagnosis and individualised therapy is of great importance.

Metabolism is a critical event in maintaining growth and other functions in cancer cells. Tumour cells require high metabolism rates to sustain active proliferation and other biological events that require a large amount of energy. In contrast with non-cancerous mammalian cells, cancer cells are always in a hypoxic environment due to their fast growth and the limited oxygen supply. The metabolism in tumour cells shifts from oxidative phosphorylation to aerobic glycolysis, known as the Warburg effect.[4] For aerobic glycolysis, pyruvate kinase (PK) is a rate-limiting enzyme[5,6] that catalyses dephosphorisation of phosphoenolpyruvate to pyruvate to yield one molecule of ATP. This molecule has four isoforms: PK type M1 (PKM1), PK type M2 (PKM2), PK type L, and PK type R; PKM1 is found in muscle and brain tissue, PKM2 in embryonic and tumour cells, PK type L in the liver and kidneys, and PK type R in red blood cells.

In tumour tissues, the expression of PKM2 is upregulated since it is essential for the process of aerobic glycolysis.[5] Polypyrimidine tract-binding protein 1 (PTBP1) regulates alternative splicing of PK, resulting in PKM2 (and can lead to switching of the PKM isoform from PKM1 to PKM2), which is expressed in a broad range of human cancers.[7] PKM2 expression was shown to be involved in early tumourigenesis,[8] and an increase in PKM2 level correlates with tumour size and stage.[9] This high expression indicates that active aerobic glycolysis occurs and regulates numerous cell functions in these cells.[10] Moreover, PKM2 expression is strongly correlated with gastric cancer differentiation; differentiated types of cancers express more PKM2 protein than undifferentiated cancers.[11] Clinical studies have demonstrated that PKM2 is released into the bloodstream and levels of PKM2 in ethylenediaminetetraacetic acid (EDTA) plasma samples are increased in gastrointestinal cancers.[12]

However, the clinical and prognostic implications of PKM2 as a marker for gastric cancer are still unclear. Abnormal glucose metabolism in cancers can be used as a target for cancer treatment. Several agents targeting glycolysis have been reported to have significant cytotoxicity for cancer cells in preclinical studies, with some of these agents having advanced into clinical studies.[13] However, the use of these agents for the treatment of gastric cancer has not been reported. The objective of this literature-based review was to determine whether PK could be used as a marker to diagnose and monitor gastric cancer.

METHODS

A PubMed search was conducted focussing on PK in gastric cancer. The PubMed database was searched with the terms ("stomach neoplasms"[MeSH Terms] OR ("stomach"[All Fields] AND "neoplasms"[All Fields]) OR "stomach neoplasms"[All Fields] OR ("gastric" [All Fields] AND "cancers"[All Fields]) OR "gastric cancers" [All Fields]) AND (("pyruvic acid"[MeSH Terms] OR ("pyruvic"[All Fields] AND "acid"[All Fields]) OR "pyruvic acid"[All Fields] OR "pyruvate"[All Fields] OR "pyruvates" [MeSH Terms] OR "pyruvates" [All Fields]) AND "phosphotransferases"[MeSH Terms])".

Table 1: The main results of selected studies.

Study	Sample	Conclusion
Sugiyama et al.,[15] 2016	20 gastric cancer tissues, adjacent non-tumour tissues, and gastric mucosal epithelial cells; 3 human gastric cancer cell lines.	• Expression levels of miR-133b were downregulated in gastric cancer cells ($p<0.005$). • Gastric cancer cells transfected with miR-133b presented downregulation of PTBP1 expression and switching of PK isoform from PKM2 to PKM1, which resulted in suppression of tumour growth.
Zhang et al.,[16] 2016	18 gastric cancer tissues and 10 superficial gastritis specimens (controls); 4 cell lines (2 adenocarcinoma cell lines, 1 normal gastric cell line, and 1 gastric carcinoma cell line).	• miR-128b was downregulated in gastric cancer tissues ($p<0.01$). • PDK1 is a direct target of miR-128 resulting in a reduction of PDK1 expression and induction of apoptosis.
Tang et al.,[17] 2016	20 gastric cancer specimens and 20 adjacent non-tumour tissues; 4 gastric cancer cell lines and 1 normal gastric cell line.	• miR-let-7a expression was downregulated in gastric cancer tissues ($p=0.0002$). • miR-let-7a overexpression inhibited the expression of PKM2 ($p<0.05$) leading to decreased proliferation of gastric cancer ($p=0.038$), suppressed migration and invasion of gastric cancer cells ($p<0.001$), and inhibited tumour growth ($p<0.05$).
Gao et al.,[18] 2015	124 gastric adenocarcinoma samples and 124 non-neoplastic gastric mucosa samples.	• PKM2 was upregulated in cancer tissues. • Upregulation of PKM2 significantly correlated with both nodal metastasis and advanced TNM stage. • Overexpression of PKM2 was associated with decreased median survival durations (42.6 months versus 80.7 months) ($p<0.001$).
Lin et al.,[19] 2015	58 gastric cancer tissues specimens, 2 cell lines, and 2 mice.	• MACC1 enhanced the Warburg effect by upregulating the expression of PDK1-4, which facilitates the replenishment of energy in gastric cancer cells to overcome metabolic stress and keep growing.
Wang et al.,[20] 2013	3 human gastric cancer cell lines (2 with expression of E-cadherin and 1 without expression of E-cadherin).	• Gastric cancer cell lines showed a high level of PKM2 expression. • In cell lines with PKM2 knockdown, decreases in proliferation and E-cadherin expression levels were observed. • PKM2 attenuates cell motility and invasion when E-cadherin is present, like in the early stages of gastric cancer. When the tumour progresses, the lack of E-cadherin induces an aggressive function of PKM2 in the tumour.
Yin et al.,[21] 2013	142 tissue blocks from patients with advanced gastric cancer who underwent curative surgery.	• The level of PKM2 correlated with tumour size ($p=0.0001$), depth of invasion ($p=0.002$), and lymph node metastasis ($p=0.036$). • The expression levels of PKM2 and VEGF in gastric cancer tissues correlated ($p<0.01$). • In patients with advanced gastric cancer, PKM2 and VEGF expression were significant prognostic factors. • The 5-year overall survival rate in patients expressing lower levels of PKM2 and VEGF was significantly better than in those expressing higher levels of both proteins ($p<0.01$).
Hur et al.,[22] 2013	152 tissue blocks from patients with gastric adenocarcinoma who underwent curative surgery; 6 gastric carcinoma cell lines and 1 non-cancerous kidney cell line.	• Positive staining for HIF1α was significantly correlated with positive PDK1 expression ($p=0.029$). • PDK1 staining significantly correlated with tumour invasion ($p=0.020$), the presence of positive metastatic lymph node ($p=0.040$), and larger tumour size ($p=0.006$). • PDK1 expression was significantly correlated with the disease-free and overall survival rates. • The cell lines with the highest level of PDK1 expression demonstrated decreased responsiveness to 5-FU treatment ($p<0.001$).
Lim et al.,[23] 2012	60 gastric cancer tissues, 19 non-cancer gastric tissues, and a tissue microarray from 368 gastric cancer patients.	• PKM2 levels were increased in primary gastric cancers ($p<0.001$) and in differentiated type cancers ($p<0.001$). • PKM2 expression strongly correlated with gastric cancer differentiation ($p<0.001$) but was not related to stage ($p=0.811$). • PKM2 expression correlated with shorter overall survival ($p<0.042$) in signet-ring cell cancers.
Kwon et al.,[24] 2012	Cell lines and 188 tumour samples.	• There was a positive correlation between PKM2 expression and the tumour size. • The cell lines transduced with PKM2 shRNA presented growth inhibition due to the apoptotic pathway. • There was a positive correlation between PKM2 and *Bcl-xL*: inhibition of PKM2 downregulated the *Bcl-xL* gene, resulting in increased apoptosis and reduced cell growth.
Tsukamoto et al.,[25] 2010	22 gastric carcinoma tissues and 5 non-neoplastic gastric epithelia; 3 gastric cell lines and a miR microarray platform covering a total of 470 human miRNA.	• miR-375 was the most downregulated miR in gastric carcinoma, especially in the early phase of tumourigenesis. • Ectopic expression of miR-375 caused reduction of PDK1 expression and induced apoptosis in gastric carcinoma cells.

Table 1 continued.

Study	Sample	Conclusion
Kumar et al.,[26] 2007	A total of 56 references relevant to tumour PKM2.	• Tumour PKM2 can be quantified in blood with a specificity of 90–95% at a diagnostic cut-off value of 15.0–17.5 U/mL and in stool with a specificity of 83–95% at a cut-off value of 3.33–4.00 U/mL. • The stability of tumour PKM2 is best in EDTA plasma for 24 hours at room temperature and is not influenced by any mechanical stress. • Tumour PKM2 can be elevated in benign conditions. • Its diagnostic accuracy was comparable to CA72-4 in gastric cancer.
Schneider et al.,[27] 2005	122 gastric cancer patients and 53 controls (persons without any malignant disease).	• At 95% specificity, tumour detection was possible by the best single marker (CA72-4) in gastric cancer in 61% of cases. • A tumour marker panel increased sensitivity significantly in gastric cancers to 81% with CA72-4 and tumour PKM2 ($p<0.001$). • Adding a third marker further improved the sensitivity only marginally. • The highest sensitivity of 91% was seen in gastric cancer patients with distant metastasis by using the fuzzy classification and the markers CA72-4 and PKM2.
Zhang et al.,[12] 2004	54 patients with confirmed gastric cancer and 20 healthy volunteers.	• The mean tumour PKM2 concentration allowed a significant discrimination of gastric cancer (26,937 U/mL) from controls (10,965 U/mL) ($p<0.05$). • In gastric cancer, tumour PKM2 showed a sensitivity of 50.47%, while CA72-4 showed a sensitivity of 35.37%.
Schneider and Schulze,[28] 2003	122 gastric cancer patients and 76 control persons without any malignant disease.	• In gastric cancers, the sensitivity of tumour PKM2 (57.0%) and CA72-4 (60.7%) were comparable and higher than CA19.9 (45.5%) and CEA (23.8%).
Yoo et al.,[29] 2004	11 human gastric carcinoma cell lines and 2 cell lines resistant to cisplatin or 5-FU.	• PKM2 showed a decrease in both activity and expression in cisplatin-resistant cell lines compared to parental cell. • When PKM2 expression and activity were suppressed by administration of antisense oligonucleotide, the cells displayed increased drug resistance. • PKM2 activity showed a positive correlation with cisplatin sensitivity ($p=0.044$).
Hardt et al.,[30] 2003	15 patients with colorectal cancer, 9 patients with gastric cancer, 3 patients with inflammatory bowel disease, and 15 controls.	• Compared to healthy subjects, samples of patients with inflammatory bowel disease or colorectal tumours did not show a statistically significant difference. • In contrast, 80% of the patients with gastric cancer had elevated PK levels in their stools ($p=0.005$).
Hardt et al.,[31] 2003	8 patients with colorectal adenomas, 49 healthy controls, 9 patients with colon cancer, 7 with rectal cancer, and 5 with gastric cancer.	• Concentrations of faecal tumour PKM2 were pronounced in colorectal cancer patients compared to the other groups.
Spellman and Fottrell,[32] 1973	Human placentae and biopsy specimens from carcinomas of the lung, stomach carcinoma, and carcinoma of jejunum, and PK purified from 5 tumours: muscle, liver, stomach, intestine, and lung.	• Human placental PK is different from other human tissues, but it is similar to tumour PK.

CA: cancer antigen; CEA: carcinoembryonic antigen; EDTA: ethylenediaminetetraacetic acid; FU: fluorouracil; HIF1α: hypoxia-inducible factor 1-alpha; miR: microRNA; PDK: pyruvate dehydrogenase kinase; PK: pyruvate kinase; PKM1: pyruvate kinase isoform M1; PKM2: pyruvate kinase isoform M2; PTBP1: polypyrimidine tract-binding protein 1; shRNA: short hairpin RNA; TNM: tumour, node, metastasis; VEGF: vascular endothelial growth factor.

A total of 32 articles were initially collected. Twelve articles were subsequently excluded from this review: 6 because the studied molecules were not PK, 2 because the studied tissues were not gastric tissue, 1 because the article was not related to gastric cancer, and 3 because they were not written in English. Ultimately, 20 studies were included in the analysis.

RESULTS

The tumour PKM2 isoform has been shown to be present not only in plasma but also in faeces, indicating that PKM2 may serve as a potential marker for screening colorectal and gastric cancers in high-risk individuals.[14] The main results of the selected studies are listed in Table 1.[12,15-32]

DISCUSSION

MicroRNA (miRNA) are small non-coding RNA molecules, 18–25 nucleotides in length, that bind to the 3' region of target messenger RNA and induce silencing of protein expression.[33-35] miRNA play important roles in a variety of processes, such as cell development, apoptosis, and cell proliferation.[33,34,36] Dysregulation of miRNA is involved in many diseases and most miRNA modulate tumour suppressor genes in various types of cancers.[33,34]

miR-133b was initially considered to be a muscle-specific miRNA involved in the development of skeletal muscle, myoblast differentiation, and myogenic-related diseases; however, a wider expression of miR-133b was found in diverse tissues.[37] miR-133b plays an important role in non-muscle-related disease, such as Parkinson's disease, cardiac failure, and cancer;[38] expression of miR-133b is downregulated in many types of cancers.[39]

Sugiyama et al.[15] concluded that miR-133b was significantly downregulated in cell lines and in gastric cancer tumour tissues compared with normal cells and tissues, respectively. Furthermore, the ectopic expression of miR-133b markedly inhibited cell proliferation through the induction of autophagy. These findings indicate that miR-133b acts as a tumour suppressor miRNA through the perturbation of the Warburg effect in gastric cancer cells. The authors also proved that, in gastric cancer cell lines transfected with miR-133b, the expression of PTBP1 was markedly downregulated, the PKM isoform expression was switched from PKM2 to PKM1 for a short duration, and the tumour growth was suppressed. The authors suggested that PTBP1 could be a target molecule for the development of anti-cancer drugs.[15]

miR-128 includes miR-128a and miR-128b,[40] and their aberrant expression was observed in many malignant tumours, but the function of miR-128b in gastric cancer is not yet known. Zhang et al.[16] found that miR-128b was downregulated in gastric cancer tissues and cell lines, suggesting that it might negatively modulate the carcinoma progression. The results showed that overexpression of miR-128b decreased cell proliferation, inhibited cell viability by arresting them in G0 or G1 phase (the proportion increased by approximately 10%; $p<0.05$), suppressed invasion, and accelerated apoptosis (the rate increased 6.5–8.8-fold) through inactivation of the Akt/nuclear factor-κB (NF-κB) axis by targeting pyruvate dehydrogenase kinase 1 (PDK1).

miR-let-7a plays a role in cell differentiation, apoptosis, proliferation, and metabolism,[41] and its levels are low in different human cancers; downregulation is associated with cancer aggressiveness.[42] Tang et al.[17] concluded that miR-let-7a was highly downregulated in gastric cancer tissues and cell lines, and its overexpression resulted in the significant decrease in cell proliferation rate, colony formation, migration, invasion, and tumourigenicity. Suppression of cell growth, migration, and invasion of gastric cancer cells was achieved by downregulating the expression of PKM2. Coexpression of PKM2 and miR-let-7a could rescue a tumour inhibited by miR-let-7a, indicating that PKM2 is the target of miR-let-7a in gastric cancer. Nevertheless, the specific mechanism by which miR-let-7a affects the expression of PKM2 was not clear. In addition, Gao et al.[18] found that PKM2 was overexpressed in gastric cancer and expression correlated with nodal metastasis, advanced tumour, node, metastasis (TNM) stages, and poor prognosis.

E-cadherin plays a critical role in maintaining epithelial integrity, and the loss of E-cadherin affects the adhesive repertoire of a cell. This molecule is also a tumour suppressor with a frequently reduced or silenced expression, and its re-expression can induce morphologic reversion.[43] Epidermal growth factor receptor (EGFR) proteins enhance cell motility and at least two distinct intracellular signalling pathways are required for EGFR-mediated cell motility: the pathways using phospholipase C-γ and the mitogen-activated protein kinase pathway.[44] Wang et al.[20] demonstrated that the knockdown of PKM2 decreased the activity of E-cadherin and enhanced the EGFR signalling pathway in the cell lines that were positive for E-cadherin expression. However, in the undifferentiated gastric carcinoma cell line, which lacked E-cadherin expression, PKM2 promoted cell migration and invasion.

The major factors that determine the prognosis of gastric cancer include lymph node metastasis,

depth of tumour invasion, and tumour size. Tumour angiogenesis plays a critical role in metastisation and tumour growth. Any increase in a tumour mass must be preceded by an increase in the microvasculature to deliver nutrients and oxygen to the tumour and remove products of tumour metabolism. Without new blood vessels, most tumours would never grow beyond 1–2 mm in diameter and would remain localised to the primary site.[45] EGFR is one of the most important regulators of angiogenesis. Yin et al.[21] concluded that PKM2 and vascular endothelial growth factor expression were positively correlated with the prognosis of advanced gastric cancer.

Hur et al.[22] concluded that glucose transporter-1 and PDK1 expression were significantly associated with tumour progression, although only PDK1 expression was an independent prognostic factor for patients who received 5-fluorouracil (FU) adjuvant treatment. Treatment with dichloroacetate, a PDK1 inhibitor, reduced lactate production and increased responsiveness to 5-FU in cell lines that expressed high levels of PDK1.

Lim et al.[23] showed that PKM2 was overexpressed in gastric cancers both at the messenger RNA and protein levels compared to non-cancerous gastric tissues. Moderately and well-differentiated adenocarcinoma showed significantly higher expression of PKM2 (60.0% PKM2-positive cells) in contrast with signet-ring cell cancers, which showed 17.7% PKM2-positive cells. NF-κB is a transcription factor that controls the expression of proteins involved in the regulation of immune response and cell survival.[46] Deregulation of NF-κB signalling is associated with oncogenesis and cancer malignancies because its activation increases the expression of many genes involved in cell proliferation, metastasis, angiogenesis, and anti-apoptosis pathways.[47]

Kwon et al.[24] identified *PKM2* as an overexpressed gene in gastric cancer patients at both the transcriptional and protein levels and showed that *PKM2* expression level affected the survival of gastric cancer cells. High *PKM2* expression was associated with poor prognosis in gastric cancer patients. PKM2-mediated NF-κB stabilisation may underlie the molecular basis for increased survival in gastric cancer cells, in part by regulating the expression of *Bcl-xL*, an apoptosis-related gene.

miR-375 has been reported to be downregulated in head and neck,[48] pancreatic,[49] and hepatocellular carcinomas,[50] but its function in cancer remains to be determined. In gastric carcinoma cells, Akt phosphorylation has been reported to promote cell survival and act against apoptotic stimuli.[51] Tsukamoto et al.[25] found that miR-375 was the most downregulated miRNA from a microarray with 470 human miRNA. Re-expression of miR-375 in gastric carcinoma cell lines resulted in induction of apoptosis and reduced cell viability. Exogenous miR-375 suppresses the expression of PDK1, resulting in decreased phosphorylation of Akt in gastric carcinoma cells. Decreased expression of miR-375 may provide a survival advantage to gastric carcinomas via activation of the PDK1/Akt survival pathway. Downregulation of miR-375 results in enhanced expression of 14-3-3ζ and provides a survival advantage to gastric carcinoma.

Currently used tumour markers have a low sensitivity for detecting cancer and their role is limited to detecting recurrence after surgery or monitoring the response to treatment. Tumour PKM2 can be measured in the stool and in the bloodstream by a highly sensitive enzyme-linked immunosorbent assay (ELISA). Kumar et al.[26] compared different tumour markers and concluded that the diagnostic accuracy of tumour PKM2 was comparable to CA72-4 in gastric cancer; using a combination of these tumour markers increased the diagnostic strength. Schneider et al.[27] found that the sensitivity of tumour PKM2 (57%) was comparable to CA72-4 (61%) in gastric cancer, and the two-marker combination increased the sensitivity to 81% ($p<0.001$). For the discrimination of malignant versus non-malignant diseases, the fuzzy classificatory (a mathematical procedure for a non-invasive analytical method) increased sensitivity by 20% compared to the best single marker in gastric cancer.[27] In another study, Schneider and Schulze[28] demonstrated that the discrimination power of tumour PKM2 was superior in colorectal, gastric, and oesophageal cancers without distant metastasis, whereas Zhang et al.[12] concluded that the sensitivity of tumour PKM2 in the diagnosis of gastric cancer was

lower than that in the diagnosis of colorectal cancer, although it was higher than that of CA72-4. In addition, Hardt et al.[30] found that stool samples of gastric cancer patients had elevated PK concentrations compared to healthy controls and inflammatory bowel disease patients. In another study by Hardt et al.,[31] the authors found a significant difference in faecal tumour PKM2 concentrations between cancer patients and controls, and the highest concentrations were observed in colorectal cancer cases. Lastly, studies also showed that the sensitivity of faecal tumour PKM2 was 73.00% and the specificity was 78.00%;[52] whereas the sensitivity and specificity of serum PKM2 in gastric cancer was 50.47% and 90.00%, respectively,[12] and 66.70% and 88.90% in colorectal cancer, respectively.[53]

Chemotherapies for advanced gastric cancer, usually containing cisplatin and/or 5-FU, have response rates of 20–40%, with between 6 and 12 months of median survival.[54] However, cancer cells can be unresponsive to drug treatment at the outset of therapy (intrinsic resistance) or they may become unresponsive after exposure to the chemotherapy agent (acquired resistance).[55] Yoo et al.[29] linked PKM2 activity and cisplatin-resistance mechanisms. They observed that cisplatin resistance correlated with decreased levels of PKM2 protein and activity in human gastric carcinoma cell lines and that lowering PKM2 expression through antisense transfection increased cisplatin resistance.

CONCLUSION

Measurement of PKM2 or PDK1 in the bloodstream or stools of patients could be a good marker for gastric cancer when analysed in combination with CA72-4; these markers are related to tumour burden, proliferation, invasion, differentiation, and lower survival. Once the diagnosis of gastric cancer is set, the combination of these two biomarkers could help monitor the response to treatment, as well as detect progression or relapse. PKM2 is also associated with poor prognosis, so patients with higher levels of PKM2 at diagnosis could have lower survival rates. A consideration for future development is that targeting PKM2 could become a new therapeutic approach for the treatment of gastric cancer patients.

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Slowing Progression of Airway Diseases by Smoking Cessation and Reducing Infections

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Abstract

The prevalence of respiratory diseases, including asthma and chronic obstructive pulmonary disease, has increased in recent decades, placing a significant burden on healthcare systems and economies around the world. As these diseases are largely incurable, the aim of treatment is to control symptoms and improve quality of life. Aside from stopping smoking and reducing biomass fuel exposure, arguably the most effective strategy in the long-term management of chronic respiratory diseases is the prevention or control of respiratory infections via vaccines and antimicrobial agents. By preventing these infections or reducing exposure to some of the major risk factors, we can reduce further lung damage in these patients, thereby slowing disease progression. This review looks at maintaining long-term respiratory health in patients with asthma and chronic obstructive pulmonary disease, primarily through smoking cessation, reducing exposure to allergens and air pollutants, and infection control.

INTRODUCTION

The health, economic, and social burden of chronic respiratory diseases, including asthma and chronic obstructive pulmonary disease (COPD), is significant and increasing.[1,2] In 2014, the average prevalence rate of asthma among adults across European Union (EU) countries was just over 6%, and for COPD it was 4%.[3] It is estimated that COPD alone causes around 3 million deaths each year, and it is predicted to become the third biggest killer worldwide by 2030.[4] To combat the increasing burden of these diseases, the Global Initiative for Asthma (GINA) was launched in 1993, followed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 1997. In recent reports (published in 2018),[5,6] both GOLD and GINA advise three main approaches for preventing premature death from airway disease: early and accurate diagnosis, preventing progression, and prolonging survival.

While accurate diagnosis is important for the optimal management of respiratory disease, patients with COPD and asthma can present with similar symptoms (e.g., cough and dyspnoea), making differential diagnosis difficult. To reflect the overlap of the common disorders that cause airflow limitation, the diagnosis of asthma-COPD overlap (ACO) has been coined.[6] Similarly, bronchiectasis and COPD share many pathophysiological characteristics and may coexist as an overlap syndrome similar to ACO.[7] However, due to the paucity of randomised controlled trials (RCT) to guide treatment strategies, the GINA and GOLD guidelines currently recommend treating these overlapping diseases according to the dominant phenotype.

Smoking is the number one risk factor for developing COPD and is estimated to be responsible for ~80% of all COPD cases, although other factors (e.g., occupational exposures, a history of infections like tuberculosis, and genetic factors such as alpha-1 trypsin deficiency) can play a role.[8] For asthma, the main risk factor in atopic individuals is exposure to allergens;[9] for those with non-atopic asthma, smoking, air pollutants, occupational exposures, advanced age, lower socioeconomic status,[9] and genetic factors may be involved.[10] Therefore, policies aimed at reducing exposure to some of these risk factors (e.g., to tobacco smoke, allergens, and air pollutants) may reduce the incidence of COPD and asthma in future.

As there is currently no cure for COPD or asthma, the interventions recommended by GINA and GOLD aim to control symptoms and improve patients' quality of life. The use of bronchodilators and anti-inflammatory drugs can improve symptoms such as shortness of breath. However, other interventions besides β2-agonists, antimuscarinics, and inhaled corticosteroids can reduce the symptomatic burden and prevent acute exacerbations. While the aetiology of these exacerbations can vary, the major causes are bacterial or viral infections.[11,12] As the frequency and severity of acute exacerbations in patients with COPD and asthma is linked to disease progression (i.e., accelerated decline of lung function), reduced quality of life, and increased morbidity and mortality,[13] infection control is a key consideration for maintaining long-term respiratory health. This review focusses on maintaining long-term respiratory health in patients with COPD or asthma through smoking cessation, reducing exposure to allergens and air pollutants, and infection control.

SMOKING CESSATION AND RESPIRATORY HEALTH

In 1998, the UK government published a white paper on tobacco, stating that smoking is the single greatest preventable cause of ill health.[14] Smoking is associated with disease progression, increased mortality, and worsening of symptoms in both asthma and COPD.[15] Asthmatic smokers have more severe symptoms and increased risk of exacerbations.[16] In addition, asthmatic smokers have an elevated propensity to develop COPD or ACO.[17] Smoking tobacco also directly increases the risk of subsequent respiratory infections, which again are associated with disease progression.[18]

As smoking is a modifiable risk factor, smoking cessation is strongly indicated by both GOLD and GINA as a clinical strategy to improve outcomes for patients with COPD or asthma.[5,6] Smoking cessation in asthmatics improves lung function, symptoms, treatment outcomes,

and quality of life.[19,20] For COPD, smoking cessation is the only intervention that has been shown to slow the decline in the forced expiratory volume in one second (FEV_1),[21] reduce all-cause mortality,[22] and improve all four core symptoms of COPD (chronic cough, chronic phlegm production, wheezing, and shortness of breath).[23] The earlier the smoking cessation, the greater the impact on lung function.[21] In addition, the use of e-cigarettes to help smokers with COPD reduce their cigarette consumption was recently found to lead to marked improvements in annual exacerbation rates.[24] However, the use of e-cigarettes remains controversial, as their overall safety profile is not well defined.

Smoking cessation with pharmacological support ranks as one of the best value-for-money options among the available COPD interventions (£2,000 per quality-adjusted life year [QALY], according to the COPD value pyramid).[25] Due to its cost-effectiveness, the European Respiratory Society (ERS) recommends that smoking cessation treatment be integrated into the management of a patient's respiratory condition and pharmacological treatment should be included in combination with behavioural support and counselling.[26] It is also strongly advised that those with asthma and COPD avoid environmental smoke exposure. This includes encouraging parents or carers of those with respiratory diseases not to smoke.[5,6]

AVOIDING ALLERGENS, BIOMASS FUEL SMOKE, AND OTHER AIR POLLUTANTS

Exposure to allergens and environmental pollutants (e.g., indoor and outdoor air pollution and occupational exposure) may also cause or exacerbate COPD or asthma.[27] Indeed, it is increasingly becoming evident that indoor air pollution from biomass fuel exposure (i.e., the burning of wood, charcoal, etc., for cooking and heating) is an important risk factor for non-smoking-related COPD.[28] To reduce the detrimental health effects of air pollutants, the Royal College of Physicians (RCP) and Royal College of Paediatrics and Child Health (RCPCH) presented a number of key recommendations in their report titled 'Every breath we take: the lifelong impact of air pollution'.[29] However, further research is required to determine the benefits of some of these strategies (e.g., the use of mixed fuel for cooking or heating) on respiratory diseases.[28]

The 2018 GINA report states that avoiding outdoor allergens (e.g., by closing windows and doors when pollen counts are high) may be beneficial in those with asthma (Table 1 [Evidence D]).[6] While avoiding indoor allergens is not recommended as a general strategy in asthma, reducing exposure to mould can improve asthma symptoms (Table 1 [Evidence A]).[6] In addition, the use of non-polluting heating and cooking sources (and adequate ventilation) is recommended for patients with asthma and COPD (Table 1 [Evidence B]).[5,6] Finally, the most recent GOLD report advises that patients with COPD should avoid continued occupational exposure to potential irritants (Table 1 [Evidence D]).[5]

VACCINATION AGAINST RESPIRATORY INFECTIONS

As viruses and certain bacteria have been shown to contribute to acute exacerbations in both asthma and COPD, vaccination is a key strategy to slow progression of these diseases.[30] The current evidence for the use of vaccines in the management of COPD and asthma is summarised in Table 1.

Influenza Vaccine

There is solid evidence for a beneficial role of the seasonal influenza vaccine in patients with COPD. A systematic review found seasonal influenza vaccination reduces the number of acute exacerbations in those with COPD, presumably triggered by influenza virus infection.[32] A retrospective cohort study in Spain also found that influenza vaccination reduced the risk of severe exacerbations in the year following immunisation, especially in those with severe COPD.[2] Indeed, according to the COPD value pyramid, influenza vaccination is considered to be more cost-effective (£1,000 per QALY) than smoking cessation with pharmacological support (£2,000 per QALY) or the use of long-acting β2-agonists (£8,000 per QALY).[25] However, a Cochrane review of the evidence from available RCT demonstrated no significant reduction in the frequency of

asthma exacerbations following influenza vaccination.[33] Indeed, according to the latest GINA report, seasonal influenza vaccination is not expected to reduce the frequency or severity of asthma exacerbations.[6] Although there remains some debate regarding efficacy in reducing exacerbations, the European Centre for Disease Prevention and Control (ECDC) currently recommends annual influenza vaccination for all patients with COPD, as well as those with moderate-to-severe asthma.[31]

Haemophilus influenzae Vaccines

The nontypeable (non-capsulated) form of *Haemophilus influenzae*, a common bacterium in the nasopharynx, can cause considerable inflammation when present in the lower airways of patients with COPD, thereby worsening symptoms.[34] While there are vaccines available against *H. influenzae* b that aim to prevent systemic infections (e.g., bacteraemia, meningitis) caused by the capsulated form of *H. influenzae*, unfortunately they have no activity against the non-capsulated forms of *H. influenzae* implicated in COPD exacerbations. An oral nontypeable *H. influenzae* (NTHi) vaccine has been developed; however, a Cochrane review demonstrated oral NTHi vaccination does not significantly reduce the number and severity of exacerbations in patients with recurrent exacerbations of chronic bronchitis or COPD.[35] Nonetheless, there are new vaccines under development for NTHi (as reviewed by Murphy[36]) that may offer improved outcomes for those with COPD in future.

Pneumococcal Vaccines

Streptococcus pneumoniae (pneumococcus) colonisation occurs more frequently in the airways of those with COPD than healthy individuals, and colonisation is associated with a higher incidence of exacerbations.[37] Those with asthma are also at increased risk of pneumococcal disease,[38] which in turn increases the risk of wheezing.[11] Therefore, injectable polyvalent pneumococcal vaccines may provide some protection against morbidity in those with COPD or asthma.

Table 1: Vaccination recommendations in patients with asthma or chronic obstructive pulmonary disease.

Vaccine	Administration details	Patients with asthma	Patients with COPD
Influenza	Annually for all those at risk.[31]	Recommended in adults with moderate-to-severe asthma (Evidence D);[6] not expected to reduce the frequency or severity of asthma exacerbations (Evidence A).[6]	Recommended in all adults with COPD to reduce serious illness and death (Evidence B).[5]
Pneumococcal (formulations available which contain different serotypes)	Single dose, but schedules vary between EU countries.[31]	Insufficient evidence to recommend routine pneumococcal vaccination in people with asthma (Evidence D).[6]	Recommended in adults with COPD (PPSV23 vaccine), if not previously vaccinated.
Pertussis or whooping cough (diphtheria, tetanus, and pertussis: DTaP or Tdap)	One dose every 10 years, but schedules vary between EU countries.[31]	Recommended in all children during their first year of life (and a booster between 5–10 years of age).[30] Recommended in all adults aged 19–64 years, including those with asthma who have not received the vaccination in their lifetime.[30] Insufficient evidence of its efficacy in preventing asthma exacerbations.	Recommended in all children during their first year of life (and a booster between 5–10 years of age).[30] Recommended in all adults aged 19–64 years, including those with COPD who have not received the vaccination in their lifetime.[30] Insufficient evidence of its efficacy in preventing COPD exacerbations.

Evidence A: randomised clinical trials (rich body of data); Evidence B: randomised clinical trials (limited body of data); Evidence C: non-randomised trials (observational studies); Evidence D: panel consensus judgement.

COPD: chronic obstructive pulmonary disease; EU: European Union; FEV_1: forced expiratory volume in one second.

In addition to the current vaccination schedule (i.e., in all babies, and in those aged 19–64 years as required), pneumococcal vaccines are recommended for patients aged ≥65 years and for younger COPD patients with significant comorbidities.[5] Clinical efficacy and recommendations for the use of pneumococcal vaccines in chronic respiratory disease (especially COPD) patients were recently reviewed by Froes et al.[39] who concluded that: "[...] pneumococcal and influenza vaccinations can prevent community-acquired pneumonia and acute exacerbations in COPD patients, while pneumococcal vaccination early in the course of COPD could help maintain stable health status."

As there is currently insufficient evidence to recommend routine pneumococcal vaccination in those with asthma (Table 1 [Evidence D]; panel consensus judgement),[6,40] the UK Joint Committee on Vaccination and Immunisation (JCVI) currently only recommends immunisation for adults with COPD; asthma is not an indication, unless requiring continuous or frequent use of systemic steroids.[41] However, next-generation vaccines against *S. pneumoniae* are being developed that could have a significant impact on respiratory health.[42] Chalmers et al.[43] recently called for more research into community-acquired pneumonia, which receives poor funding relative to its high disease burden, and for which contemporary data are required.

Pertussis Vaccine

Associations between *Bordetella pertussis* infection (whooping cough) and COPD have also been found; however, there is no association between *B. pertussis* infection and severity of COPD.[44] In those with asthma, *B. pertussis* infection has been shown to lead to worsening of symptoms.[30] Nonetheless, due to a paucity of clinical trials in the UK on the effectiveness of pertussis vaccination in patients with COPD or asthma, use of the vaccine in adults is currently only recommended for pregnant women to protect the newborn baby; specific guidelines for adults with chronic respiratory disease are lacking.[30]

Problems with Coverage

Although pertussis vaccination coverage is high across EU countries, vaccination rates for influenza and pneumococcal disease vary. For example, influenza vaccination coverage in clinical risk categories for all EU member states varies from 28.7–78.7%.[31] In Spain, pneumococcal vaccination coverage was found to be less than acceptable in COPD patients aged >40 years (67.5% men and 60.4% women).[45] The low vaccination rates may be due to a lack of awareness of infection-associated complications, anti-vaccination campaigns,[46] and other factors (such as the recent ruling by the Court of Justice of the European Union [CJEU] in a compensation case, in which a man claimed that a vaccination for hepatitis B caused his multiple sclerosis).[47]

Some propose that vaccination will be most cost-effective in those that are at high-risk; indeed, a model was recently developed that can predict the risk of exacerbations in COPD and could be used to identify those who would most likely benefit from vaccines.[48] However, as childhood respiratory infections are also a key risk factor for developing chronic respiratory diseases such as asthma and COPD,[8] it is still important to improve vaccination rates overall across Europe through the collaborative efforts of healthcare providers and public health initiatives.

ANTIMICROBIALS IN THE PROPHYLAXIS AND TREATMENT OF RESPIRATORY INFECTIONS

Evidence for the benefit of antimicrobial agents for treating or preventing asthma exacerbations is limited. A recent RCT found no clinically significant benefit from azithromycin treatment in asthma attacks.[49] Therefore, based on the limited data, prescribing antibiotics for preventing or treating asthmatic exacerbations is not recommended, unless there is strong evidence of lung infection.[6]

The use of antibiotics in preventing or managing COPD exacerbations is also controversial. Although COPD exacerbations are often treated with short-term (5–7 days) antibiotic use, there are conflicting data from clinical trials concerning

the efficacy of this treatment. Indeed, a systematic review of 16 RCT in people with acute COPD exacerbations found antibiotics had no statistically significant benefit on mortality or hospitalisation duration.[50]

In terms of prophylactic use in stable disease, a 2013 Cochrane review of seven RCT concluded that the use of continuous prophylactic antibiotics (azithromycin, erythromycin, and clarithromycin) reduced the number of exacerbations in patients with COPD.[51] Nonetheless, despite its benefits, it is estimated that >80% of COPD patients have risk factors for adverse complications from azithromycin therapy.[52] Indeed, a study found those with chronic lung disease receiving long-term azithromycin therapy had an increased risk of hearing impairment and a 2.7-fold increased risk of colonisation with resistant bacteria relative to placebo controls, although azithromycin-treated patients had a lower risk of new colonisation (risk ratio: 0.55) and were less likely overall to be prescribed antibiotics for treatment (risk ratio: 0.63) compared with controls.[53] In addition, a post-hoc analysis found patients with exacerbations requiring both antibiotic and steroid treatment, older patients, and those with milder disease (based on GOLD stage) may benefit from azithromycin treatment, while active smokers showed less benefit (i.e., no reduction in exacerbations of COPD was seen in this group).[54] Therefore, antibiotic treatment regimens should be individualised and guided by the severity of symptoms, risk of exacerbations, and side effects.

Azithromycin (and potentially other antimicrobial agents) may be acting as immunomodulators. In smokers with emphysema, azithromycin therapy was shown to cause changes in the composition of the lower airway microbiota, which in turn increased the levels of bacterial anti-inflammatory metabolites, and subsequently decreased the levels of host-produced inflammatory chemokines (tumour necrosis factor-α, interleukin [IL]-12 p40, IL-13, and CXCL1).[55] However, further investigation of the underlying mechanisms of the therapeutic effects of azithromycin in COPD is required.

Inhaled antibiotics have also recently gained attention for the prevention of COPD exacerbations. Patients with severe COPD colonised by multidrug-resistant *Pseudomonas aeruginosa* who were treated with nebulised tobramycin (twice daily for 14 days) showed reductions in local inflammation and bacterial load, with a concurrent 42% reduction in severe exacerbations after 6 months of treatment.[56] The use of inhaled antibiotics for managing COPD exacerbations, however, requires further study.

Overall, at present, there is no Grade A evidence (RCT; rich body of data) on which GOLD or GINA base their recommendations regarding the role of antibiotics in preventing or treating infections during COPD and asthma exacerbations.[5,6]

The Importance of Antimicrobial Stewardship

Despite the aforementioned lack of evidence, it is estimated that >80% of COPD patients in secondary care and 50% of patients managed in primary care are treated with antibiotics.[57] This is concerning as antibiotic use in patients with COPD may be contributing to antimicrobial resistance (AMR). To respond to the increasing threat of AMR, antimicrobial stewardship programmes have been developed in many countries worldwide (e.g., the UK One Health Report[58]).

Antimicrobial stewardship programmes aim to educate healthcare professionals to use an evidence-based approach when prescribing antibiotics, which will change according to the patient's individual situation. Unfortunately, guidelines regarding the prescription of antibiotics in COPD and asthma are, at present, poorly followed, particularly in the case of recurrent exacerbations.[59] For example, it is recommended that sputum diagnostics should be implemented in patients with early treatment failure or repeated exacerbation as soon as antibiotic treatment is started; however, at present, sputum cultures are only performed in a minority of cases.[59] Moreover, expectorated sputum specimens are subject to significant contamination by oropharyngeal bacteria, making it difficult to distinguish between true pathogens and contaminants in cultured specimens. Newer diagnostic tests are being developed to describe the entire population of bacteria in bronchoalveolar lavage specimens, as there is mounting evidence that

exacerbations are actually due to a pathological change in the composition of the population of microbes at the site of infection, rather than the presence of a single pathogen.[57]

To help reduce the impact of frequent antibiotic use in COPD on AMR, more research is required to identify which patients would benefit from antibiotics (such as the study by Han et al.[54] on daily azithromycin use). Developing novel or more effective vaccines to prevent airway infections may also help reduce the emergence of multidrug-resistant strains. This was highlighted in a 2016 report commissioned by the UK Prime Minister,[60] which concluded that we are not moving anywhere near fast enough to develop or use vaccines to reduce AMR. Preventing airborne infections in healthcare settings is another key strategy. The infection protection and control interventions available to prevent airborne infections include handwashing, healthcare worker vaccination, vaccination of patients, patient isolation, antiviral treatment, and the use of facemasks.[61] Blanco et al.[61] estimated that combining the above interventions in a bundle had the potential to reduce influenza transmission in a hospital by up to 50%.

Table 2: Interventions other than bronchodilators and inhaled steroids to reduce the burden of asthma and chronic obstructive pulmonary disease.

Intervention	Patients with asthma (GINA)[6]	Patients with COPD (GOLD)[5]
Smoking cessation	Strongly encourage smokers to quit at every visit; provide access to counselling/smoking cessation programmes (Evidence A).	Smoking cessation interventions should be actively pursued in all patients with COPD (Evidence A).
Avoiding environmental smoke exposure	Strongly encourage people with asthma to avoid environmental smoke exposure (Evidence B). Advise parents or carers of children with asthma not to smoke and not to allow smoking in rooms and cars that their children use (Evidence A).	Strongly encourage people with COPD to avoid environmental smoke exposure.
Avoiding allergen exposure	Not recommended as a general strategy in asthma but limited evidence of clinical benefit for sensitised patients (Evidence A). Removing household mould or dampness can reduce asthma symptoms and medication use in adults (Evidence A). Limited evidence of clinical benefit for patients sensitised to dust mites and/or pets (Evidence B). Limited evidence of benefit of avoiding outdoor allergen exposure (i.e., when pollen and mould counts are highest) by closing windows and doors, remaining indoors, and using air conditioning for sensitised patients (Evidence D).	Recommend advising patients to avoid continued exposure to potential irritants, if possible (Evidence D).
Avoiding biomass fuel smoke exposure	Recommend sources of pollutants to be vented outdoors, and encourage the use of non-polluting heating and cooking sources (Evidence B).	Recommend sources of pollutants to be vented outdoors, and encourage the use of non-polluting heating and cooking sources (Evidence B).
Avoiding outdoor air pollutants	Limited evidence of benefit of avoiding strenuous outdoor activity during unfavourable outdoor conditions (e.g., air pollutants and very cold weather) (Evidence D).	Recommend advising patients to avoid continued exposure to potential irritants, if possible (Evidence D).
Influenza vaccination	Recommended annually, or at least when advised, for the general population (Evidence D).	Recommended annually, or at least when advised, for the general population to reduce serious illness and death (Evidence B).
Pneumococcal vaccination	Insufficient evidence to recommend its use (Evidence D).	Recommended to reduce the incidence of community-acquired pneumonia in COPD patients aged <65 years with an FEV_1 <40% predicted and in those with comorbidities (Evidence B).
Pertussis (whooping cough) vaccination	Recommended in all adults who have not been previously vaccinated.	Recommended in all adults who have not been previously vaccinated.

Table 2 continued.

Intervention	Patients with asthma (GINA)[6]	Patients with COPD (GOLD)[5]
Antibiotics as prophylactics	Not routinely recommended.	Insufficient evidence to provide recommendation.
Antibiotics to treat exacerbations	Not routinely recommended.	May be recommended (for a course of 5–7 days) when indicated (e.g., increased dyspnoea, sputum volume and purulence, or in those requiring mechanical ventilation); the risks and benefits must be assessed on an individual basis.
Preventing airborne infections in healthcare settings	Recommend handwashing, healthcare worker vaccination, vaccination of patients, patient isolation, antiviral treatment, and the use of facemasks.	Recommend handwashing, healthcare worker vaccination, vaccination of patients, patient isolation, antiviral treatment, and the use of facemasks.

Evidence A: randomised clinical trials (rich body of data); Evidence B: randomised clinical trials (limited body of data); Evidence C: non-randomised trials (observational studies); Evidence D: panel consensus judgement.

COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second; GINA: Global Initiative for Asthma; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

CONCLUSIONS

As chronic airway diseases are largely incurable, prevention through reducing exposure to risk factors such as tobacco smoke is our best strategy. However, as summarised in Table 2, we also have a number of cost-effective interventions to reduce the symptomatic burden of these disorders to maintain the long-term respiratory health of patients with COPD or asthma. These strategies include smoking cessation; reducing exposure to allergens, pollutants, and biomass fuels; and infection control through vaccines and antibiotics. Regarding antibiotics, it is becoming increasingly necessary for all healthcare workers to make better choices regarding their use in respiratory infections, by relying on new diagnostics and adhering to antimicrobial stewardship programmes. As we enter a post-antibiotic era, the discovery of new and improved vaccines, and appropriate evidence-based vaccination schedules, is crucial for preventing not only the development of COPD and asthma but also the number of exacerbations and disease progression. By implementing the strategies reviewed here, we can support the vision of the World Health Organization (WHO) to create a world where all people can breathe freely.

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Initiatives to Improve Safety of Oral Anticancer Agents Delivered by Community Pharmacists

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Abstract

With the recent growth in oral anticancer agents (OAA), pharmacists working in the community have recognised the urgent need to develop safe and effective systems to administer and manage these drugs. For community pharmacists, education regarding OAA can be challenging, with a number of international surveys showing that many believe they have received inadequate education regarding OAA and feel uncomfortable educating their patients about these drugs. Patients prescribed OAA have also reported feeling unsupported, and this lack of support could lead to both under and overadherence to OAA, with an impact on efficacy and adverse events. Poor adherence can result in disease progression, treatment complications, reduced functional ability, and premature death.

The current review, written by international authors from Europe, North America, and East Asia, set out to identify worldwide initiatives to support community pharmacists working with patients taking OAA. The authors identified one project, the Oral Anticancer Therapy – Safe and Effective initiative, that was developed in Germany in 2011 to aid community pharmacists in their interactions with patients prescribed OAA. The initiative, which has been rolled out across Germany, includes the creation of training programme content that can be delivered at regional meetings and monographs, which can be downloaded to educate both community pharmacists and their patients about individual OAA. As part of the Empowering Patients to Improve Health Care for Oral Chemotherapy (EPIC) programme, the European Society of Oncology Pharmacy (ESOP) has extended the German initiative to Slovenia and Estonia, with plans to launch the scheme in additional European countries in the autumn of 2018. Ultimately, it is hoped that better support of cancer patients in the community will improve adherence to OAA.

GROWTH IN ORAL ANTICANCER AGENTS

In recent years, oncology therapies have undergone a paradigm shift from being delivered mainly as intravenous (IV) chemotherapy in hospitals and outpatient clinics to oral anticancer agents (OAA) taken at home. This change has had wide-ranging repercussions for the workload and care delivery model of pharmacists, in particular those working in the community.

In 1995, only six OAA were available, but by 2007 >12 were in use and between 2015 and 2017 23 were approved by the U.S. Food and Drug Administration (FDA).[1,2] A similar picture holds for Europe, with two OAA being approved by the European Medicines Agency (EMA) between 2001 and 2003 compared to 16 OAA between 2015 and 2017 (Figure 1). The way OAA have revolutionised oncology can be appreciated by considering that 25 OAA were approved by the FDA between 2011 and 2014, compared with 18 cancer IV agents during the same period.[3] It has been shown that the total drug expenditure in the USA on targeted therapies (almost exclusively OAA) increased from 26% in 2010 to 40% in 2016,[4] and it is estimated that 25–30% of all haematological oncology drugs currently in development are orally administered, small molecules.[5] OAA, which can be delivered as tablets, capsules, or a liquid, range from traditional endocrine and cytotoxic therapies to biological therapies targeted at cell surface proteins or mechanisms specific to cancer biologic pathways.

As a direct result of the growth in the number of OAA available, cancer patient management has evolved from a process that was controlled and monitored by clinicians and nurses in hospitals and outpatient clinics, to one that involves patients and their caregivers having the majority of treatment responsibility.[6] For patients, the convenience of oral therapy offers the potential to improve quality of life; with OAA, patients need fewer hospital and outpatient appointments; thus, they are required to spend less time away from their work and families. An additional advantage is that there is no requirement for IV access, which can cause complications such as extravasations, venous sclerosis, infections, and injection site reactions.[7]

Many studies have suggested that patients prefer OAA to IV therapy;[8-11] however, although patients can feel empowered by taking direct responsibility for managing their treatment, this responsibility can prove overwhelming for sick patients who lack reliable support from families and friends.[12] Candidates for OAA need to be well-motivated, have good health literacy, and be able to manage complex regimens. There is also a requirement for a healthy food intake and gut function, with minimal nausea and vomiting, since the bioavailability of oral agents is greatly affected by diet.[13]

The introduction of OAA to the community also offers advantages to health services, including the potential for cost savings as a result of reduced hospital admissions or outpatient infusions. Indeed, a UK time and motion study showed that switching from IV chemotherapy to OAA allowed a seven-fold increase in the number of patients treated.[14] In addition, surveys have shown that both patients and healthcare professionals perceive OAA to be safer than IV cancer therapy.[15,16]

However, patients often do not fully appreciate that OAA can have life-threatening levels of toxicity and often incorrectly believe that they are similar to vitamins or antibiotics. OAA treatment can result in fatalities; for example, in the UK, a National Patient Safety Agency (NPSA) alert on oral anticancer medicines was issued in 2008 following three deaths and 434 safety incidents that occurred between November 2003 and July 2007.[17] Many stakeholders do not fully appreciate that in addition to the hazards posed to patients, there are risks for family caregivers and healthcare personnel involved in handling OAA. Studies have found that up to two-thirds of staff handling these medications showed measurable amounts of the agent in their urine.[18,19]

In the hospital and clinical settings, safety systems for IV chemotherapy have been well-developed, with prescribers using electronic order sets, pharmacists verifying and preparing treatments, and nurses educating patients and delivering therapy. This infrastructure creates the ability to assess toxicity and adjust therapy at the point of delivery.[20] In contrast, when patients are prescribed OAA in the community, safety and support systems may not be in place.

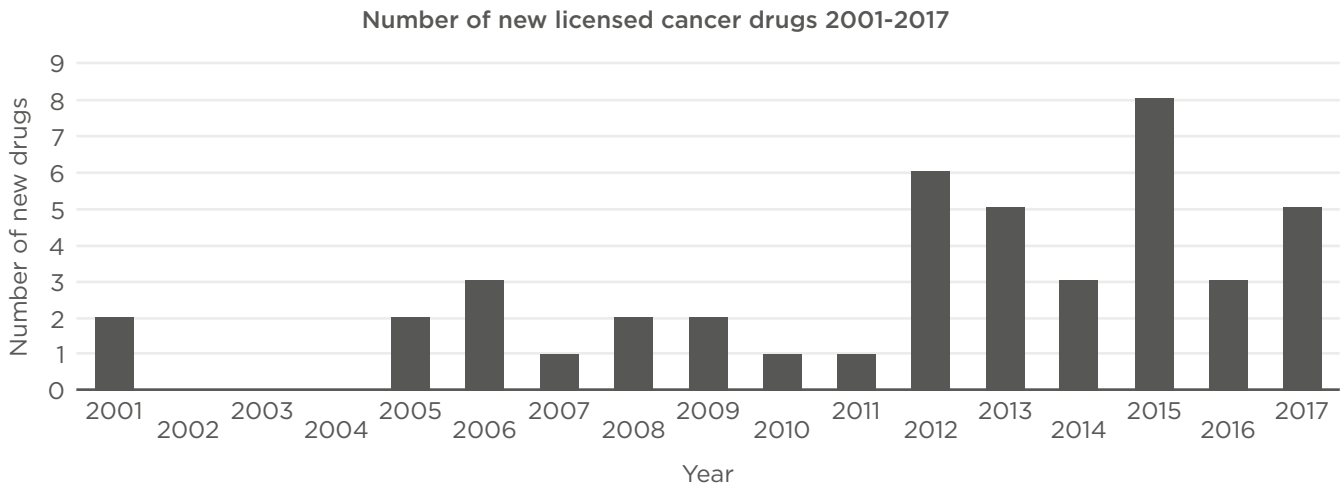


Figure 1: Graph showing the year that different oral anticancer agents were licensed in Europe. Courtesy of Klaus Meier.

2001: capecitabin, imatinib; 2005: anagrelide, erlotinib; 2006: dasatinib, sorafenib, sunitinib; 2007: lenalidomide; 2008: lapatinib, nilotinib; 2009: gefitinib, thalidomide; 2010: pazopanib; 2011: abiraterone; 2012: axitinib, crizotinib, ruxolitinib, tegafur-kombi, vandetanib, vemurafenib; 2013: afatinib, dabrafenib, enzalutamide, regorafenib, vismodegib; 2014: cabozantinib, ibrutinib, idelalisib; 2015: carfilzomib, ceritinib, cobimetinib, lenvatinib, nintedanib, olaparib, panobinostat, trametinib; 2016: osimertinib, palbociclib, tipiracil; 2017: alectinib, ixazomib, ribociclib, tivozanib, venetoclax.

Prescriptions may be completed by community pharmacists who often have not been provided with adequate information about the patient's disease, weight, height, test results, concurrent medications, or dietary habits. One concern is that when cancer therapies are taken orally there may be a lack of independent checking, with safeguards routinely adopted for IV chemotherapy not used for oral chemotherapy.

A study from the USA reported the results of a survey of OAA safety practices at 42 USA cancer centres and found that information required on a prescription, such as diagnosis, cycle number, prescriptions checked by other clinicians, calculation of body surface area, or dose per m^2 per body surface area, was variable.[21] The study showed that 10 centres had no formal process for monitoring adherence and 10 centres reported at least one serious adverse event in the previous year.[21] Additionally, a Canadian study showed that a total of 57 systematic checks were identified for IV chemotherapy but only six for oral chemotherapy.[22]

When patients take medications at home there are risks of poor adherence. One study involving 119 patients taking OAA for various types of cancer showed that of the 33 patients who were nonadherent to OAA, 20 were overadherent and 13 underadherent.[23] Without comprehensive toxicity management and adherence programmes, oncologists may be assessing responses to therapy without knowing the degree of treatment adherence. If clinicians are unaware that OAA are not being used as prescribed, disease progression may be inappropriately attributed to lack of efficacy and clinicians may change treatments unnecessarily.[24]

Undoubtedly, there is an urgent need to develop effective systems to improve the quality and safety of OAA delivery and management. Pharmacists, in particular those working in the community, face challenges to update their knowledge, reduce medication errors, provide safe handling of drugs, manage side effects, and deliver education to patients and their caregivers. Internationally, there is a need to develop infrastructures to ensure that patients receiving OAA in the community receive standards of care that are equivalent to those receiving IV therapy in hospitals.

INCREASED ROLE FOR COMMUNITY PHARMACISTS

The healthcare settings in which OAA are dispensed vary widely around the world. In the UK, Belgium, and Poland, all OAA are dispensed by hospital pharmacy departments, while in other European countries, including Germany, France, Spain, Ireland, Slovenia, Croatia, and Estonia, community pharmacists play an important role in OAA delivery (Figure 2). In the USA, the dispensing of OAA is largely restricted to specialist pharmacies that employ pharmacists with disease-specific expertise in oncology,[25] and, in Canada, a hybrid system exists in which cancer agencies dispense in the western provinces and community pharmacies dispense in the eastern provinces.[26] In Japan, both hospital and community pharmacists provide OAA to patients.[27]

Educational systems regarding OAA are starting to be put in place for hospital pharmacists. For example, in the UK, hospital pharmacists prescribing OAA are required to have received accreditation from the British Oncology Pharmacy Association (BOPA), and in the USA the Board of Pharmacy Specialties (BPS) provides board certification for oncology pharmacists. In Japan, the completion of an outpatient chemotherapy pharmacy certification programme, known as the accredited pharmacists of ambulatory cancer chemotherapy (APACC) and offered to both hospital and community pharmacists, is one of the requirements for receiving OAA healthcare reimbursement fees.[27]

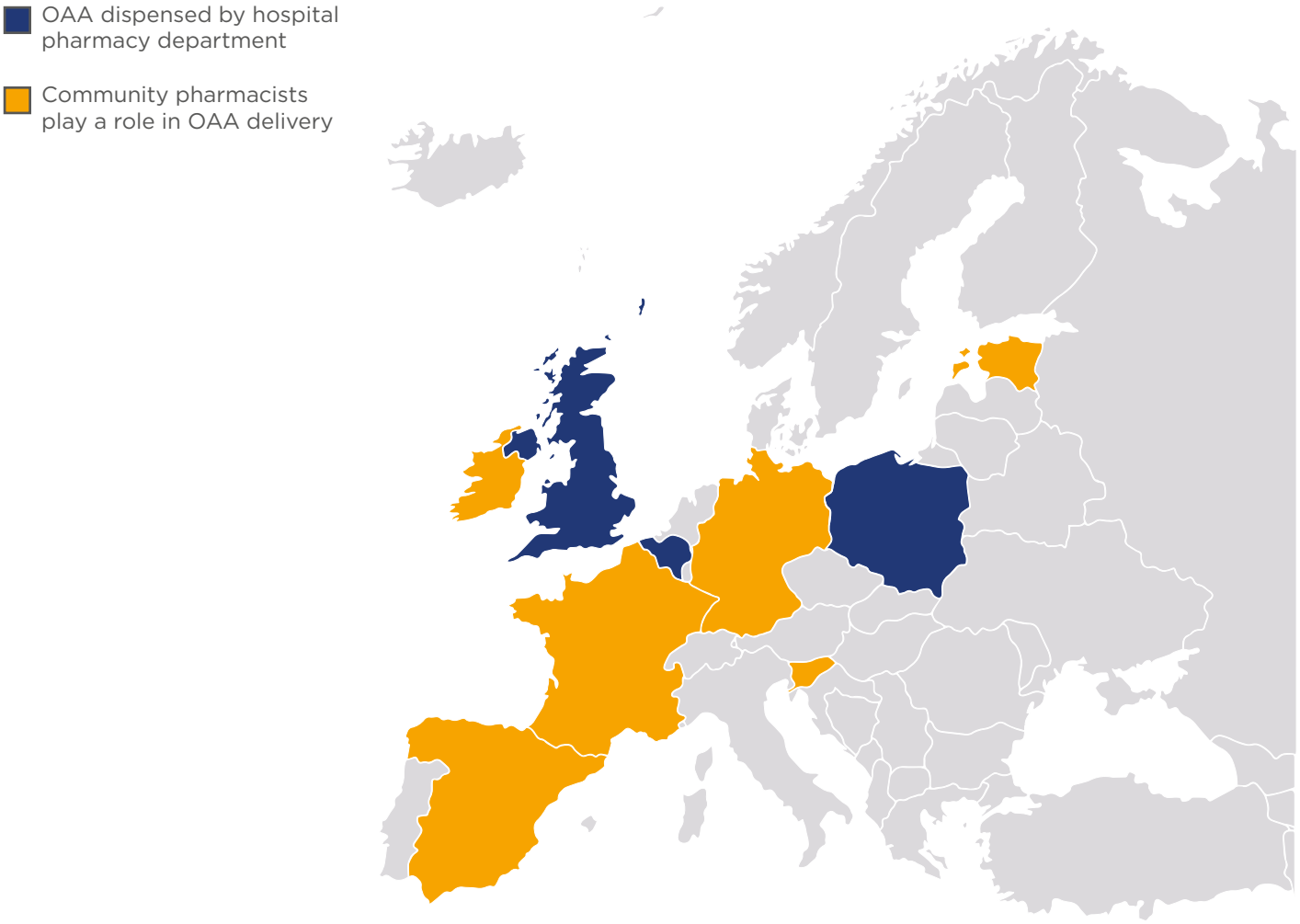


Figure 2: Map of Europe showing the countries in which oral anticancer agents are dispensed by hospital pharmacy departments and in which community pharmacists play an important role.

OAA: oral anticancer agents.

Standards for OAA have also been produced by a range of organisations, including The American Society of Clinical Oncology (ASCO) and the Oncology Nursing Society (ONS),[28] the Japanese Society of Pharmaceutical Oncology (JASPO), and the Canadian Association of Provincial Cancer Agencies (CAPCA).[29] These outline a set of expectations and frameworks for individual healthcare providers in relation to oral chemotherapy, and JASPO has published guidance on oral therapy collaborations between hospital and community pharmacists.[30] However, universal guidelines for managing adherence to oral chemotherapy have yet to be established.

Most undergraduate pharmacy curriculums only touch on OAA briefly, with the result that most community pharmacists have gaps in their knowledge. With OAA becoming more widely used, community pharmacists, even in European countries where the prescription and control of OAA is controlled by hospital teams, should be educated in the field to participate in the compliance and control of the side effects of the drugs.

Surveys undertaken in a range of different countries revealed that community pharmacists felt concerned by their lack of oncology training and did not feel equipped to ensure the safe use of OAA.

A survey of 283 Japanese community pharmacists, which took place between May and June 2014, found that only 6–10% felt they had received adequate education and training regarding OAA. Furthermore, although 81% of respondents had attended at least one continuing education event relating to oncology in the past 2 years, only 54% felt comfortable dispensing OAA and 40% felt comfortable educating patients about OAA. Only two pharmacies in the survey (0.3%) had a certification related to oncology pharmacy.[27]

A similar theme emerged from Canada, with a survey of 352 community pharmacists finding that 13.6% of respondents felt they had received adequate oncology education at undergraduate level, 19.0% had attended a continuing education event relating to oncology in the past 2 years, 24.0% were familiar with the common doses of OAA, and only 9.0% felt comfortable educating patients.[31] Similarly, in Ireland, two-thirds of community pharmacists surveyed felt they did not have sufficient information available to safely dispense prescriptions for OAA and three-quarters felt the current Irish system placed patients at risk.[32]

Patients have also reported feeling unsupported; a survey by the German Society for Oncology Pharmacy (Deutsche Gesellschaft für Onkologische Pharmazie [DGOP]) of 427 patients receiving OAA from community pharmacists found that one-third of respondents had not received any advice, although around one-half would have welcomed it.[33] Taken together, such surveys demonstrate the need to develop education programmes to support community pharmacists in playing a greater role in the care of patients receiving OAA.

IMPORTANCE OF PATIENT ADHERENCE

One of the greatest concerns related to dispensing OAA in the community is that the lack of supervision will affect patient adherence, resulting in both under and overadherence, and that this will in turn reduce efficacy and increase the risk of adverse events. Adherence implies a collaborative approach to decision-making between patients and healthcare staff, while compliance has the connotation of a passive role for patients in receiving and following medical advice. Optimal adherence is achieved "if no doses are missed, no extra doses are taken, and no doses are taken in the wrong quantity or at the wrong time".[34]

Studies across a range of different disease areas have showed that approximately half of all patients do not take their medications as prescribed.[35] A systematic review undertaken between January 2003 and June 2015 showed that rates of adherence for OAA varied from 46–100% depending on patient sample, medication type, follow-up period, assessment measure, and calculation of adherence.[36] Adherence to OAA appears higher than for other medications, most likely due to cancer being perceived by patients as a life-threatening disease. The significance of nonadherence varies markedly between OAA. Taking the example of breast cancer hormone therapies, patients will be far less compromised by missed doses of

tamoxifen (half-life: 7–14 days)[37] than missed doses of the aromatase inhibitors letrozole and anastrozole (half-life: 2 days and 27 hours, respectively).[38] Poor adherence to OAA can result in unnecessary disease progression, treatment complications, reduced functional abilities, lower quality of life, and premature death.[39] For example, an imatinib study in chronic myeloid leukaemia showed that 23% of patients with suboptimal responses were nonadherent compared to 7% nonadherence with optimal responses.[40] Other studies demonstrated that tamoxifen patients who completed <70% of their prescriptions had an increased risk of death[41] and that renal cell carcinoma patients who did not adhere to axitinib and everolimus showed significant decreases in progression-free survival.[42]

Reasons for nonadherence are attributed to three main themes: personal patient factors (belief in the treatment and emotional state), treatment factors (side effects, complexity of the treatments, and costs), and healthcare provider factors (relationship with the healthcare professionals and prescribing practices).[34] Focussing on treatment factors, frequency, severity, and types of side effects of medications are all likely to affect adherence. This can be especially the case for OAA, which often have novel modes of action with rare and unexpected side effects;[43] for example, OAA can cause life-threatening side effects, including neutropenic sepsis and diarrhoea, and other side effects such as hypertensive episodes and extreme skin toxicities. Adverse drug reactions to OAA are common; a study reviewing 1,061 prescriptions for OAA at an academic outpatient cancer centre over 1 year reported that, within 90 days of initiation, 80% of patients had experienced treatment-related toxicities secondary to an OAA, with 36% classified as severe and 17% requiring hospitalisation.[43]

Comorbidities also complicate treatment. A study showed oncology patients have an average of 3.2 comorbid conditions for which they take 10–12 medications,[44] all of which having the potential to interact with OAA. An academic outpatient centre study found that, in addition to OAA, patients were prescribed a mean of 10.9 medications and had a mean of 2.1 major drug interactions.[43] Taking the example of erlotinib, drugs that increase the pH of the upper gastrointestinal tract may alter its solubility and reduce bioavailability, and inhibitors or inducers of CYP3A4 and CYP1A2 can increase or decrease plasma concentrations.[45] OAA can also interact with foods; for example, one paper reported that at least 15 OAA have clinically significant interactions with grapefruit.[46]

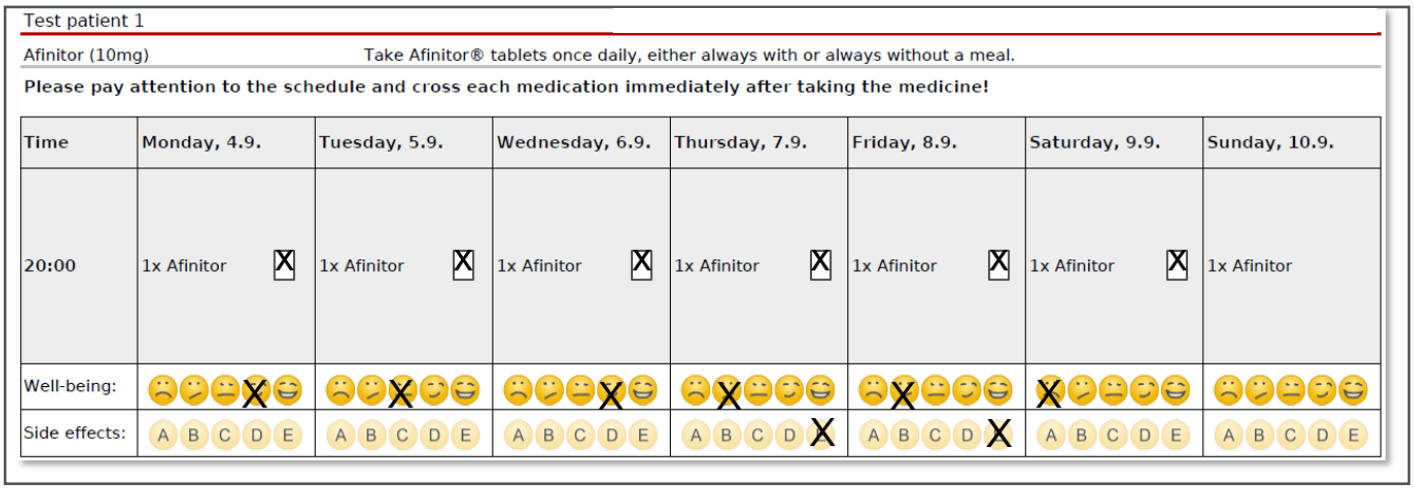
The complexity of OAA treatment can also include the cycling of medications, during which patients are required to have 'on' and 'off' days. For example, capecitabine for metastatic colorectal cancer is taken every 12 hours within 30 minutes of a meal for 2 weeks, followed by a 1-week break before a new cycle begins.[47] One study demonstrated significant associations between regimen complexity and adherence when patients on simple regimens with the same dose and frequency throughout treatment were compared to those taking complex regimens that included alternating periods on and off medications and fluctuations in the prescribed dose.[23] In the USA, the level of patient cost-sharing (where co-payments are required) has also been found to be a significant factor determining adherence to OAA, with some patients rationing medications or not having prescriptions processed. One study showed that treatment claims with cost-sharing >$500 were four-times more likely to be abandoned than claims with cost-sharing ≤$100.[48]

A systematic review exploring adherence to OAA in breast cancer patients found that the most commonly reported motivator for improving adherence was the patient–provider relationship.[49]

In Germany, the DGOP recognised that community pharmacists have the potential to serve as agents for change in improving adherence to OAA therapy.

INITIATIVES SUPPORTING COMMUNITY PHARMACISTS WORKING WITH PATIENTS TAKING ORAL ANTICANCER AGENTS

In 2011, the DGOP joined forces with the German Cancer Society (Deutsche Krebsgesellschaft) to develop tools to support community pharmacists interacting with cancer patients taking OAA.


Test patient 1

Afinitor (10mg) Take Afinitor® tablets once daily, either always with or always without a meal.

Please pay attention to the schedule and cross each medication immediately after taking the medicine!

Time	Monday, 4.9.	Tuesday, 5.9.	Wednesday, 6.9.	Thursday, 7.9.	Friday, 8.9.	Saturday, 9.9.	Sunday, 10.9.
20:00	1x Afinitor X	1x Afinitor X	1x Afinitor X	1x Afinitor X	1x Afinitor X	1x Afinitor X	1x Afinitor
Well-being:	X	X	X	X	X	X	
Side effects:	A B C D E	A B C D E	A B C D E	A B C D X	A B C D X	A B C D E	A B C D E

Figure 3: Screenshot of the online database developed by the German Society for Oncology Pharmacy (Deutsche Gesellschaft für Onkologische Pharmazie [DGOP]) as part of the Oral Anticancer Therapy – Safe and Effective initiative. Courtesy of Klaus Meier.

This shows a day-to-day record of patient wellbeing containing patient-specific information, with a calendar that patients can use to record taking their drugs, a 'smiley face' survey to show wellbeing, and opportunities to record different adverse events.

A: nausea/vomiting; B: skin reaction; C: mucositis; D: diarrhoea; E: exhaustion.

Key features of the Oral Anticancer Therapy – Safe and Effective initiative included provision of content for training programmes that are delivered by oncology pharmacy experts at regional meetings. The training programme consists of presentations developed by DGOP experts that are intended to be delivered across three separate sessions lasting a total of 8 hours:

- The first session, lasting 2 hours, covers the basics of cancer therapy, dealing with terminology, epidemiology, tumour development, and principles of cancer therapy.
- The second session, lasting 4.5 hours, covers applied oncology pharmacy and addresses individualisation of dose, adherence, and adverse drug effects and their management.
- The third session, lasting 1.5 hours, explores handling of OAA, storage, administration, handling of excreted materials, disposal of waste, and cleaning.

To date, around 3,000 German community pharmacists have attended educational sessions delivered around the country by 50 DGOP members (Klaus Meier, personal communication). In addition to organising training programmes, the DGOP has developed an online database providing key facts for the 72 OAA agents available in Germany. Community pharmacists can use these monographs both to educate themselves about the agents and to inform patients. The monographs are continually updated by a DGOP working group with the intention to further extend the monographs as more information about OAA becomes available online.

The online database can be used to provide each cancer patient with bespoke medication administration plans and a calendar to track drug administration so that the pharmacist can check for adherence when they refill medications. An important feature is the day-to-day record of wellbeing that contains a simple smiley face measurement survey showing five expressions ranging from happy (represented by a smiley face) to sad (represented by a crying face) that patients can use to document their condition on a daily basis (Figure 3). The pharmacist instructs patients that if they mark an unhappy face on two subsequent days they should make an urgent appointment to see their oncologist to adjust the dose and receive advice on how to avoid side effects. The DGOP recognise that it is important for patients to document how they feel daily, since it can be all too easy to forget adverse events

when they see their oncologists. The initiative also offers the opportunity to collect information on thousands of patients taking OAA, which has the potential to gather side effect statistics that go beyond those available in drug trials. Ultimately, the DGOP hope to collect data for use in a study to demonstrate the effectiveness of good patient support by community pharmacists.

In 2015, the European Society of Oncology Pharmacy (ESOP) started the Empowering Patients to Improve Health Care for Oral Chemotherapy (EPIC) initiative, which extended the DGOP scheme to community pharmacists working in Slovenia and Estonia; training materials and drug information monographs were translated into the local languages. At the 4th European Conference of Oncology Pharmacy (ECOP), held from 25th–27th October 2018 in Nantes, France, the plan is to roll out the EPIC scheme to other European countries; Spain, France, Belgium, Austria, Denmark, Italy, Poland, Romania, and Bulgaria have already expressed an interest. Modifications to EPIC will undoubtedly be needed for implementation in countries that have different training protocols, modes of learning, and ways of dispensing OAA to Germany.

CONCLUSION

Many community pharmacists working with cancer patients taking OAA around the world have reported that they feel unsupported and require additional education. In Germany, the Oral Anticancer Therapy – Safe and Effective initiative has achieved great success, and it is hoped that when the programme is extended to other European countries it will lead to better support of cancer patients in the community and improved adherence to OAA with an overall impact on drug efficacy.

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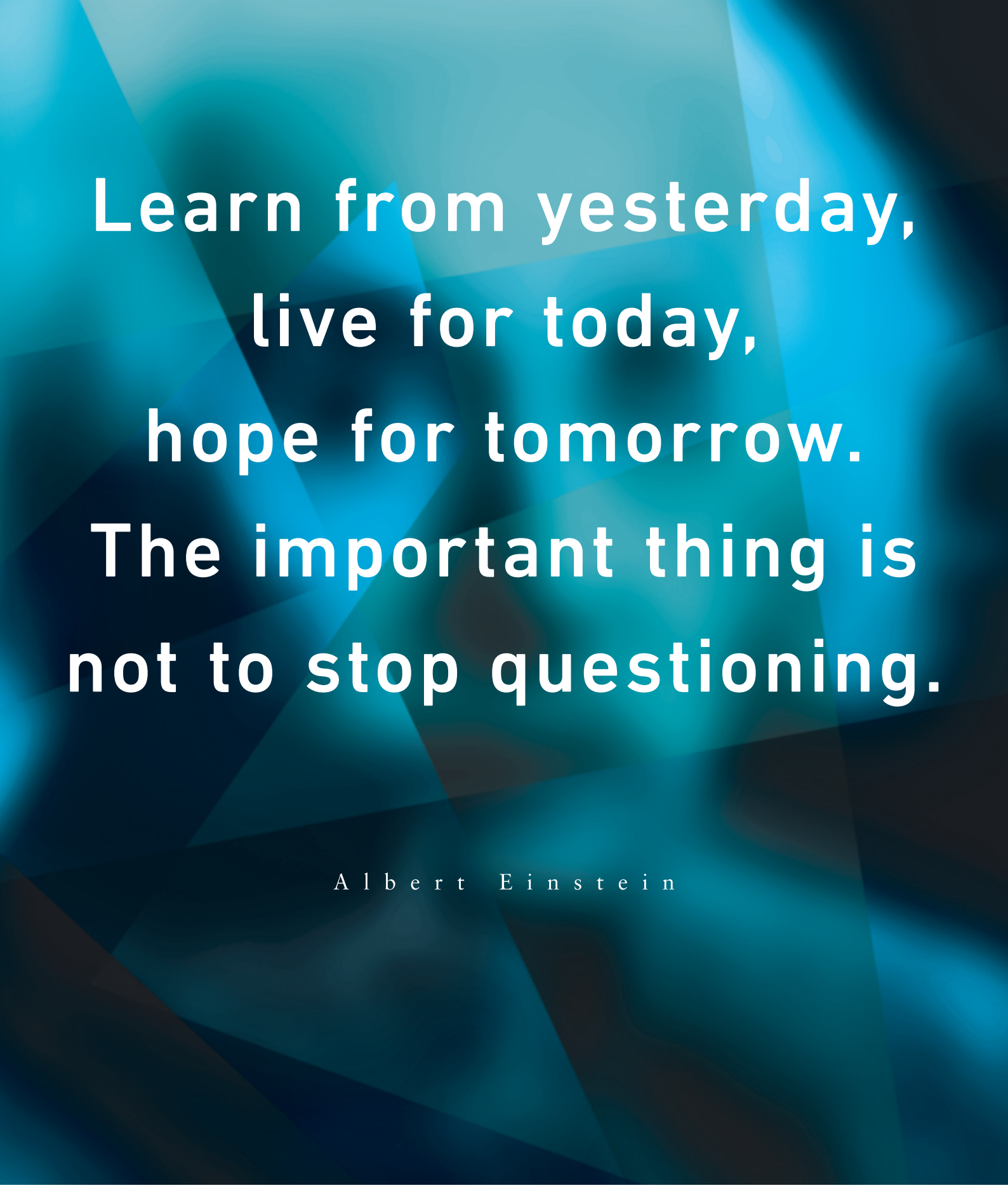

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Overactive Bladder in Children

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Abstract

Overactive bladder (OAB) is clinically defined as the presence of urinary urgency and may be associated with diurnal urinary incontinence, frequency, and enuresis, and/or constipation. In children aged 5–10 years, the prevalence is 5–12%. Association with emotional disorders is widely described in the literature. Constipation is associated with voiding symptoms because of crosstalk between the gastrointestinal tract and the urinary tract. OAB is believed to be multifactorial. Correct functioning between the pontine micturition centre, the periaqueductal grey matter, anterior cingulate gyrus, and prefrontal cortex is important for correct voiding development and the process of maturation. Patients with OAB have greater anterior cingulate gyrus activity and deactivation of the pontine micturition centre urinary inhibition process, leading to a greater frequency of bladder repletion sensation. Urotherapy is the first treatment to be initiated and aims to change behavioural patterns inthese patients. Other treatment options are anticholinergics, with oxybutynin being the most widely studied, but also described is the use of tolterodine, darifenacin, and mirabegron. Alternative treatments, such as nerve stimulation in the parasacral or the posterior tibial area, have shown improvement of symptoms in comparative studies with conventional drug treatment, and, in refractory cases, botulinum toxin A is an option. In this article, we review the pathophysiology, associated conditions, and aspects related to diagnosis and treatment of OAB.

INTRODUCTION

Overactive bladder (OAB) is a common lower urinary tract dysfunction in children, defined as the presence of "urinary urgency, usually accompanied by frequency and nocturia, with or without urinary incontinence, in the absence of urinary tract infection or other obvious pathology."[1] Clinically, urgency is commonly associated with daytime urinary incontinence and frequency; however, it may be due to voiding postponement and bladder overdistension.[2,3]

OAB is not associated with neurological and anatomical alterations of the lower urinary tract. The overall prevalence ranges from 1.5–36.4%; it is known that the peak incidence occurs between 5 and 7 years of age, with a higher prevalence in males.[4,5] The quality of life of these patients may be associated with emotional and behavioural changes, such as symptoms of depression and anxiety. Patients may have problems such as attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder.[6-8]

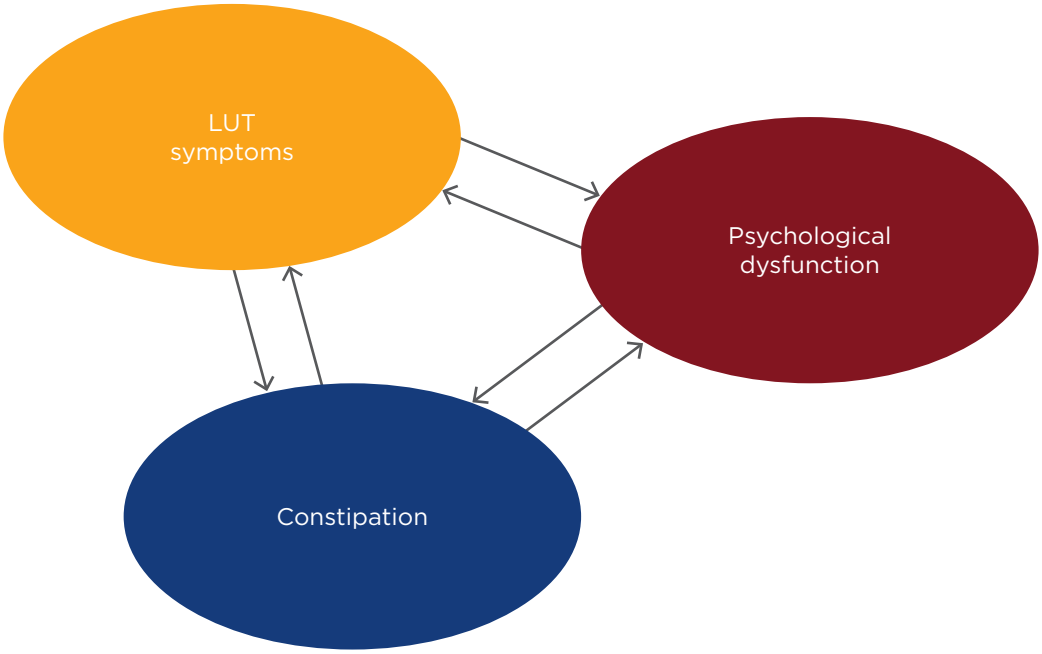

Figure 1: Multifactorial pathophysiology of bladder, bowel, and brain dysfunction.

LUT: lower urinary tract.

Although OAB can resolve itself spontaneously, urinary symptoms can persist into adulthood.[9,10] The correlation between the voiding and bowel symptoms is already very established. Children with OAB have three times more symptoms of constipation than children without urgency.[11-13] The possible explanations are that the intestinal and bladder functions are controlled by the supraspinal region,[14-17] and there seems to be crosstalk between the lower urinary tract and the rectum (Figure 1).[18] Moreover, it has been suggested that contractions of the external urethral sphincter to prevent urinary loss and simultaneous contraction of the anal sphincter may, by negative feedback, reduce the rectal transit.[14,18] Also, the hard mass of stool in the rectum could compress the bladder and modify its function.[19-21] Obese children have a higher incidence of OAB when compared to eutrophic children. Studies suggest that there may be a frontal lobe disinhibition, justifying this phenomenon.[14,15,22] Children with sickle cell and institutionalised children also have a higher prevalence of OAB.[22] This article will review the pathophysiology, associated conditions, and aspects related to diagnosis and treatment of OAB.

PATHOPHYSIOLOGY

The pathophysiology of OAB is not completely understood and is believed to be multifactorial. One theory is that the urgency and related symptoms stem from a cortical immaturity of the centres responsible for controlling urination.[14] Supporting this hypothesis, voluntary and co-ordinated urination is developed over time. In the first 2 months of life, the child voids once per hour with an intermittent flow. In this phase, the voiding is mainly controlled by the brainstem.[19,23] From the first to the third year of life, cortical inhibitory pathways and the pontine micturition centre along with periaqueductal grey matter, anterior cingulate gyrus (ACG), and the autonomic, somatic, and sensorial autonomic nervous systems are being developed, and urination becomes voluntary.[19,23] With the child's development, the prefrontal cortex would typically start to have top-down control over more primitive afferent pathways of the brain, such as the limbic and paralimbic system.

The child may also experience voiding urgency by deciding not to urinate as part of a behavioural dysfunction. Similarly to

lack of attention, voiding postponement may be the result of a behavioural dysfunction or neurophysiological immaturity. Children with oppositional defiant disorder who have difficulty following commands often refuse to go to the toilet to void or defecate, and may be affected by functional faecal incontinence.[24,25] Unfortunately, little is to be found in the literature on the neurofunctional assessment of these patients, but there seems to be hypoactivation of the prefrontal cortex.[26-28] In situations of voiding postponement, the child engages in techniques such as tightening the penis, which stimulates the dorsal nerve of the penis and thus causes the external urethral sphincter to contract and the bladder to relax.

Patients with OAB seem to have a greater ACG activity and a deactivation of the pontine micturition centre urinary inhibitory process, leading to a higher frequency of bladder repletion sensation.[3] Therefore, a hyperactive ACG may lead to a greater responsiveness to bladder filling and, consequently, to urgency.

CLINICAL HISTORY AND DIAGNOSIS

The clinical history should include family history, neuropsychomotor development, voiding and bowel training, urinary tract infection (UTI) history, school performance, and the child's behaviour and psychosocial development.[23] It was recently demonstrated that there is an association between mothers and daughters with OAB.[10,29] The intestinal constipation should be investigated and evaluated through the Bristol Stool Diary and the Roma III or IV score.[11]

OAB occurs during the bladder storage stage. However, it may be accompanied by changes in the voiding phase, such as dysfunctional voiding. In a study, the authors demonstrated that some urinary symptoms have a low correlation with more objective data.[30] Therefore, additional exams should be requested, such as urine tests, ultrasound (US) of the urinary tract, uroflowmetry, a bladder diary, and a bowel diary. The US should measure the post-void residual (PVR) and the bladder wall thickness and evaluate the rectal distension (≥3 cm suggests faecal impaction).[11] The PVR is different according to age. The PVR is considered high in children 4–6 years old if it is ≥30 mL or >21% of bladder capacity, and in children 7–12 years old if it is ≥20 mL or >15% bladder capacity.[2]

A patient with OAB has a bell-shaped or tower-shaped uroflowmetry curve, which suggests urinary urgency (Figure 2).[1] A urodynamic study should be reserved for those cases where there is failure after treatment or for patients who have signs of non-neurogenic bladder or even myogenic failure.[23,31]

The bladder diary consists of the record of frequency and urine volume and of daytime urinary incontinence for a period of 48 hours. The expected bladder capacity is calculated by [Age (years) + 1] x 30 mL. The International Children's Continence Society (ICCS) defined as increased daytime urinary frequency as ≥8 times per day and decreased daytime urinary frequency ≤3 times per day.[2] The bowel diary is made using the Bristol Stool Scale, also for a period of 7 days. Tools suchas the Dysfunctional Voiding Symptom Score (DVSS), the Vancouver Symptom Score for Dysfunctional Elimination Syndrome (VSSDES) questionnaires, and ROME III and IV should be used. The VSSDES can indicate diagnostic validity. The cut-off point is 11 with a threshold diagnostic sensitivity and specificity of 80% and 91%, respectively.[32] In the DVSS questionnaire, levels above six for girls and nine for boys are considered suggestive of lower urinary tract dysfunction.[33]

TREATMENT

Standard Urotherapy

Urotherapy is a very important measure in the treatment of OAB and is considered the first line of treatment. It consists of dietary hygiene changes regarding fluid intake, an adequate interval between urinations, adequate positioning to void, and defecation where the feet are supported on a flat surface with attention to the perineal musculature so that there is co-ordination between pelvic floor relaxation and bladder contraction.[11] Caffeine, chocolate, and citrus foods can be avoided because they may stimulate the urgency symptom, although there is a low level of scientific evidence for these measures.[3,5,11,23,34]

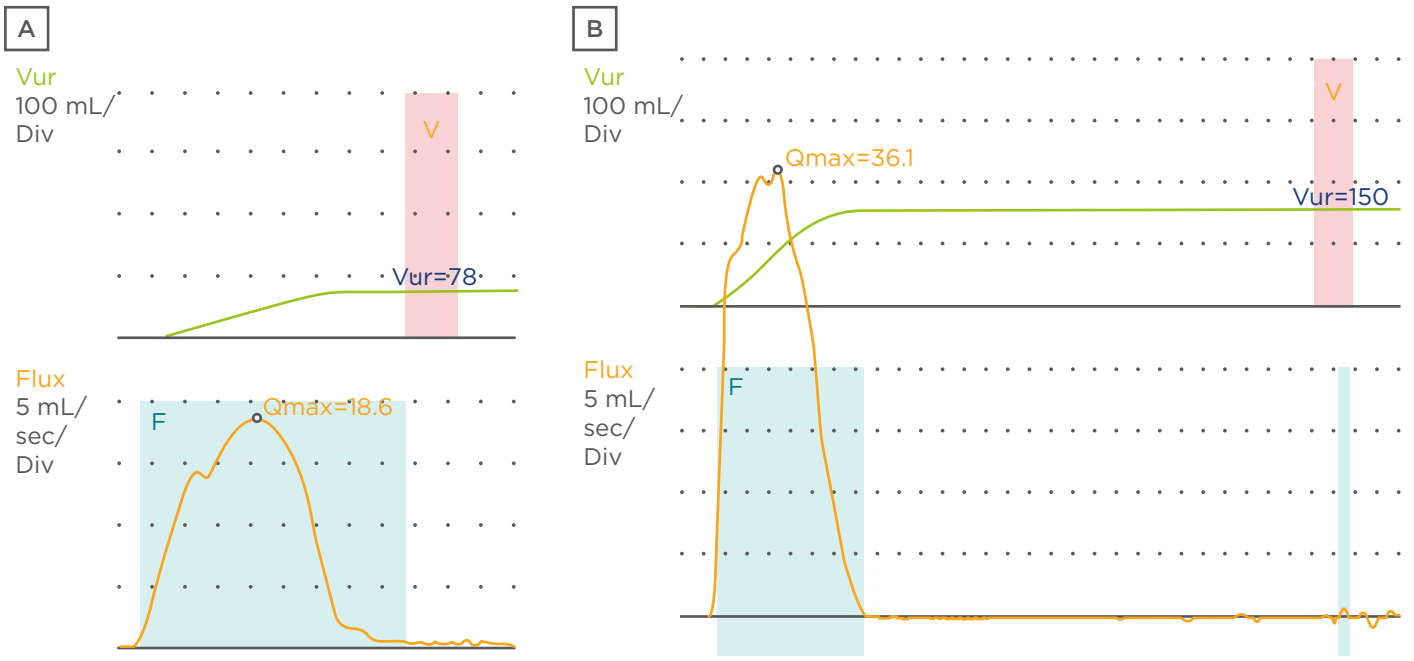

Figure 2: Uroflowmetry curves. A) Bell-shaped curve; B) tower-shaped curve.

The approach to constipation should be aggressive through hydration and fibre intake. We treat all constipated children with polyethylene glycol (PEG 3350). Initially, we treat faecal impaction at a dose of 1.0–1.5 g/kg/day (maximum dose of 100 g/day) for at least 2 months. The child should be encouraged to stay in the bathroom for 15–20 minutes at regular times.[11]

Anticholinergics

Anticholinergics are the first line of medical treatment for children who maintain OAB after urotherapy.[3] All anticholinergics may have side effects, such as dry mouth, constipation, blurred vision, urinary retention, plethora, dizziness, and delirium, especially at high doses. Oxybutynin is the most common drug and can be given as either immediate release (IR) or extended release (ER) with a recommended dose of 0.3–0.6 mg/kg.[5] van Arendonk et al.[35] retrospectively analysed 27 patients with daytime urinary incontinence who used IR oxybutynin and switched to slow ER oxybutynin. A total or partial improvement of symptoms was observed in 13 of the 27 patients with an increased voided volume (33% versus 53%; $p<0.01$) and an improved bladder capacity (55% versus 70%; $p=0.03$).[35] Intravesical administration is an alternative that allows for higher doses, with fewer adverse effects. However, this method is of little use because the children do not undergo intermittent catheterisation.[34] Tolterodine is also available in two formulas, and some studies suggest a good efficacy and good tolerability in children.[36,37] Bolduc et al.[36] demonstrated that 77% of the children using tolterodine because of non-tolerability of oxybutynin proceeded without significant side effects. Tolterodine IR is available in doses of 1 mg or 2 mg, while ER is 2 mg or 4 mg. Studies comparing the results of oxybutynin and tolterodine in children with OAB are limited; however, they suggest that tolterodine is at least as effective as oxybutynin.[38,39] Fesoterodine is the most recent long-acting antimuscarinic and is available in 4 mg or 8 mg. It is recommended for children weighing >25 kg and it is well-tolerated by children and adolescents.[40] Solifenacin is a slow-acting antimuscarinic available in a dose of 5 mg or 10 mg. Hoebeke et al.[41] demonstrated a total response rate of 85% and presence of adverse effects in 6.5% of patients with the use of solifenacin. Darifenacin is an antimuscarinic with a more selective action with less adverse effects on the central nervous system.[5] A combined therapy of two antimuscarinics (such as oxybutynin and

tolterodine) is also an option; a previous study showed that the combined drugs showed an improvement in urinary continence, and 63% of patients had moderate adverse effects, but not enough to stop the medication.[41]

Mirabegron is the β3 agonist approved for treatment of OAB in adults and has few studies of its use in children.[5] It increases bladder capacity without changing the bladder pressure and PVR and works by promoting smooth muscle relaxation through the increase of cyclic adenosine monophosphate.[3] The adverse effects commonly found in antimuscarinics have not been demonstrated in mirabegron.[5] Blais et al.[42] conducted a study in 58 children with refractory OAB and treated them with mirabegron for 11.5 months. They demonstrated a statistically significant improvement in bladder capacity and continence in these patients, with moderate adverse effects in 13% of the cases. This medication may be used as an adjuvant medication to another anticholinergic if necessary.[11]

Neuromodulation

There is a hypothesis that electrical neural stimulation (ENS) stimulates the nerves of the bladder, spinal cord, and central nervous system. The mechanism of action is still uncertain. There may be a local effect with activation of the bladder C fibres, or a spinal effect with sympathetic activation and/or parasympathetic inhibition.[43] Dasgupta et al.[44] conducted positron emission tomography/computed tomography (PET/CT) in eight patients with Fowler's syndrome and eight healthy controls to map the brain function of these patients during full bladder and the modulation during sacral nerve stimulation. In healthy controls, there was an increase in midbrain function and chromogranin A during full bladder. The Fowler's syndrome patients had no increase in brainstem activity, despite the increase in chromogranin A function in the absence of neuromodulation. The hypothesis is that neuromodulation assists in the re-establishment of voiding desire and the voiding capacity of patients.[44]

The main types of ENS are a) parasacral transcutaneous electrical nerve stimulation (TENS);[3,5,43,45] b) posterior tibial nerve stimulation (PTNS);[3,4,43,46] and c) stimulation by sacral implantation.[3,43] In the TENS, two surface electrodes are placed on S3 level, whereas in the PTNS a 34-gauge needle is placed two fingers above the medial malleolus. Quintiliano et al.[47] carried out a review study in which patients undergoing ENS showed complete resolution of OAB symptoms, urgency, and daytime incontinence in 31–78%, 25–84%, and 13–84% of patients, respectively. Randomised studies have shown TENS to be an effective method in the resolution of OAB symptoms in children and adolescents.[47,48] The rate of complete resolution of symptoms with TENS is around 60%, with a recurrence rate of 10%.[43,46]

Borch et al.[49] randomised 51 patients to three treatment groups. Group 1 received TENS plus active oxybutynin administration, Group 2 received TENS plus placebo oxybutynin administration, and Group 3 received placebo TENS plus active oxybutynin administration. All three groups experienced a positive effect in terms of decreasing urinary incontinence, incontinence severity, and urinary frequency, and improving maximum and mean bladder capacity, but in Group 3 one-third of the patients had urinary retention, which did not happen in Group 1; this signalled the importance of TENS in bladder emptying. Group 1 had a more significant improvement for daytime urinary incontinence ($p<0.01$), severity of urinary incontinence ($p<0.05$), and urinary frequency ($p<0.001$) when compared to the other groups.[49]

More recent studies have compared established treatments for OAB and TENS. Quintiliano et al.[47] analysed two methods for the treatment of OAB in children: a) oxybutynin plus sham scapular electrical therapy, and b) TENS plus placebo. They demonstrated that TENS is as effective as oxybutynin for the treatment of OAB. In the oxybutynin group, there was a decrease in voiding frequency, but the TENS group showed an improvement in constipation. Adverse effects were only present in the oxybutynin group and resulted in treatment discontinuation in 13.3% of the patients.[47]

TENS can also be used in the treatment of constipation. Veiga et al.[50] demonstrated that TENS resolved 60% of cases of constipation in patients with OAB, although it was unrelated to any improvement in OAB. Two other randomised

studies have already been completed. In one study, the participants who received 20-minute TENS sessions demonstrated increased colonic transit time compared with a control group.[51] The second study was a double-blind, controlled study that evaluated the acute effects of TENS on rectal motility and it showed that after TENS the bowel contractions improved.[52]

The PTNS approach is well tolerated in children, and studies have shown that it is an effective method in the treatment of OAB.[53] Initial studies have demonstrated an improvement in complete resolution of OAB symptoms by 56–100%.[5,54] The rate of complete resolution of symptoms was significantly higher in the TENS group compared to the PTNS group (70% versus 9%).[55] Boudaoud et al.[56] analysed PTNS versus sham treatment and concluded that, despite the improvement of urodynamic parameters in the PTNS group, there was no difference in clinical results between the two groups. Peters et al.[57] conducted a randomised, multicentre study comparing PTNS and tolterodine ER and found a similar reduction in urinary frequency, severity of urgency, and increased voiding volume.

We recommend ENS in cases of standard urotherapy failure because there are no direct side effects and a higher effectiveness against the OAB and constipation. The sacral implant is performed through percutaneous transforaminal access to stimulate the S3 nerve.[3,43] Roth et al.[58] demonstrated complete or >50% improvement in 88% of children. Of these, 63% had nocturnal enuresis, 89% improved daytime urinary frequency, and 59% had constipation.[2] However, because of the high rate of reoperations, this method is limited to patients who are refractory to the other methods.

Botulinum Toxin A

Botulinum toxin A acts in the neuromuscular junction preventing the action of acetylcholine and adenosine 5-triphosphate on the parasympathetic presynaptic terminations, promoting chemically reversible desensitisation and flaccid paralysis of the muscle.[45] The effect begins 5–7 days after injection and can last for about 6 months. The botulinum toxin A injection is a viable option for patients with refractory OAB, but few studies have been performed for non-neurogenic patients. Hoebeke et al.[59] reported a study with 21 children with non-neurogenic OAB in which 100 U of botulinum toxin were given at 15 injection sites in the bladder wall. Few adverse effects were reported, with a complete success rate of 42% in the first application, a 61% increase in bladder capacity ($p<0.001$), and 11% recurrence within 1 year.[59] It should be used in children ≥3 years of age at the dose of 5–10 U/kg. It is contraindicated in patients with peripheral motor neuropathy, neuromuscular junction disorders, UTI, uncorrected coagulopathy, and in pregnant women. The adverse effects are pain, UTI, haematuria, and autonomic dysreflexia. Around 2–9% of patients may present postprocedure transient acute urinary retention requiring clean intermittent catheterisation at this stage.[5]

CONCLUSION

OAB is a common clinical entity in children and knowledge of the neurophysiology factors of voiding provides information that helps to manage it. The diagnosis is clinical and should be complemented with a bladder diary, US of urinary tract with PVR evaluation, and uroflowmetry. Treatment should start with urotherapy, and in refractory cases TENS, PTNS, and medications can be included. If the symptoms persist, botulinum toxin A and sacral neuromodulator implants may be alternatives.

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Multiple Myeloma: Personalised Medicine Based on Pathogenesis

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Abstract

Multiple myeloma is increasingly being recognised as more than one disease, characterised by marked cytogenetic, molecular, and proliferative heterogeneity. The prognosis is widely varied, ranging from low to very high-risk, based on cytogenetic and molecular studies. Although novel agents, such as proteasome inhibitors and immunomodulators, have been developed, which have improved treatment responses and disease prognosis, multiple myeloma remains an incurable disease. Based on highly sensitive detection tools, such as gene expression profiling and next generation sequence analysis, and the understanding of the pathogenesis of multiple myeloma, many potential agents, including monoclonal antibodies, drug-conjugated antibodies, drugs targeted to molecular abnormalities, microRNA inhibitors or mimics, and immune therapies, such as chimeric antigen receptors T cells and anti-PD1 agents, can be considered personalised therapies. In this paper, multiple myeloma pathogenesis and potential molecular and immunotherapies are reviewed.

INTRODUCTION

Multiple myeloma (MM), which accounts for approximately 10% of all haematological malignancies, is a clonal, late B cell malignancy in which plasma cells (PC) accumulate in the bone marrow (BM).[1] The disease undergoes multistep transformations and its genetic landscape changes over time owing to additional events, such as somatic mutations and epigenetic and chromosomal copy-number changes; this drives the progression of MM from monoclonal gammopathy of unknown significance to symptomatic MM, often leading to an aggressive, extramedullary disease.[2] Although many novel therapeutic agents have been developed, MM still remains an incurable disease, possibly owing to its marked genetic heterogeneity.

Recently, a range of sensitive detection tools have been developed, including analysing cytogenetic abnormalities in M-phase cells and interphase fluorescent *in situ* hybridisation; molecular analysis using a microarray, which has identified a 70-gene signature of an aggressive form of the disease (gene expression profile [GEP]70);[3] and next generation sequence analysis. However, further investigation is

required to understand the pathogenesis and target genes of MM to determine the best treatment strategies for each patient.

PATHOGENESIS OF MULTIPLE MYELOMA

There are two key aspects of MM pathogenesis: the genetic lesions intrinsic to the malignant clone, and the interaction between myeloma cells and the microenvironment of the BM.[4]

Genetics of the Myeloma Cell

MM is classified as either nonhyperdiploid or hyperdiploid based on karyotype analysis. The hyperdiploid subtype accounts for 50–60% of MM patients[5] and is characterised by the presence of copy-number alterations such as trisomies of the odd chromosomes (3, 5, 7, 9, 11, 15, 19, and 21).[4] Nonhyperdiploid subtypes harbour translocations between 14q32, immunoglobulin heavy chain locus, and one of several partner oncogenes, including *MAF* (16q23), *MAFB* (20q11), *FGFR3/MMSET* (4p16.3), *CCND1* (11q13), and *CCND3* (6p21).[6]

Hyperdiploid Molecular Pathway

There are approximately 35 nonsynonymous mutations per MM case.[7] Interstitial copy-number gain associated with increased gene expression or with activating mutations in oncogenes represents another set of drivers for MM progression. For example, the amplification of 1q potentially involves more than one relevant oncogene, including *CKS1B, ANP32E, BCL9,* and *PDZK1*.[8] Interstitial copy-number gains resulting in amplification of NIK (*MAP3K14*), TAC1 (*TNFRSF13B*), and LTBR proteins can also activate the nuclear factor (NF)-κB pathway.[9]

Nonhyperdiploid Molecular Pathways

Deregulation of the G1/S Phase transition is a key early molecular abnormality in MM. Consistent deregulation of a D-group cyclin was first noted whilst studying the t(11;14) and t(6;14) translocations, which deregulated *CCND1* and *CCND3*, respectively.[10] *MAF* (t[14;16]) and *FGFR3/MMSET* (t[4;14]) upregulate *CCND2* and deregulate G1/S transition.[10]

Upregulation of NF-κB signalling is important in MM. *FAM46C, DIS3, CYLD* (16q), *BIRC2* (also known as *CLAP1*), *BIRC3* (11q), and *TRAF3* (14q32) are all tumour suppressor genes involved in the NF-κB pathway,[7-9,11] and are inactivated in MM. Furthermore, 58% of MM cases show loss of a negative cell cycle regulator, downregulation of *CDKN2C* by loss of chromosome 1p32, silencing of *CDKN2A* by methylation,[8,12] and inactivation of *RB1* as a result of loss of chromosome 13. LTB, a Type II membrane protein of the tumour necrosis factor (TNF) family, heterodimerises with LTA to generate the ligand for LTBR, which is a positive regulator of the NF-κB pathway frequently amplified in MM.[13]

The MAPK/ERK pathway is frequently deregulated, induced by genes, including *NRAS* in 24%, *KRAS* in 27%, and *BRAF* in 4% of MM patients.[14] Deregulation of the PI3K/AKT pathway, which was detected in 50% of cases, and the DEPTOR, a positive regulator, are also important.[7,15] Loss of function of *TP53*, the key gene at the 17p deletion site, is involved in the PI3K/AKT pathway and contributes to late oncogenesis of MM.[16]

The balance between apoptosis and antiapoptosis pathways contributes to cancer cell survival. t(14;18) involving the *BCL-2* locus was reported in 2–3% of MM patients, with more frequent protein level elevation.[16] *BCL2L1* and *MCL-1* are similarly upregulated in MM via the interleukin (IL)-6/STAT3 axis.[16] X-box binding protein 1 (XBP-1), a key molecule in guiding commitment and differentiation of PC, had dual roles in MM. Elevation of mRNA levels and spliced *XBP-1* correlated with poor prognosis in MM.[17] However, lack of spliced *XBP-1* mRNA was shown to allow cells to escape from proteasome inhibitor-induced death and cause treatment failure and disease relapse.[18] Sp140 showed truncating and missense mutations in MM.[14] ROBO1, a transmembrane receptor implicated in β-catenin and MET signalling, harbours a truncating mutation, and *EGR1*, which encodes the early growth response one transcription factor, carries missense mutations in MM.

Epigenetics in Multiple Myeloma

Methylation of cytosines embedded in CpG islands in the promoter region of target genes is an epigenetic mechanism of transcriptional silencing. A number of tumour suppressor

genes are hypermethylated early in MM, and increased gene methylation occurs during the process of MM progression.[19] Histone methylation and acetylation are also altered in MM.[20] The most pronounced DNA methylation change is seen in the 15% of patients with the t(4;14) translocation: these patients have increased gene-specific hypermethylation compared with other cytogenetic subgroups.[20] MMSET, which is overexpressed in this t(4;14) subgroup, mediates histone 3 lysine 36 dimethylation. The dimethylation modification leads to deregulation and global changes in histone modifications that promote cell survival, cell cycle progression, and DNA repair.[21] Bromodomains exert an epigenetic function via their direct interaction with acetylated lysine residues. The bromo and extraterminal family of bromodomains was shown to induce MYC expression in MM.[22]

microRNA

Dysregulated micro (mi)RNA expression in MM cells is associated with cytogenetic abnormalities and correlated with gene-expression changes characteristic of MM genetic subtypes and has provided the rationale for the design of new therapeutic strategies to treat MM.[1] Of the 464 miRNA analysed, 95 were shown to be overexpressed in patients with MM (Table 1).[23] These abnormally regulated miRNA target genes that regulate the cell cycle, apoptosis, survival, and cell growth; for example, the miR-17-92 miRNA cluster regulates Bcl-2;[24] miR-19a and miR-19b, which form part of the miR17-92 cluster, downregulate SOCS-1;[24] miR-29b regulates MCL-1;[25] miR-21 regulates STAT3 in an IL-6-dependent manner; and miR-125b regulates BLIMP1 and IRF4.[26] The miR106b-5 cluster, miR-181a and b, and miR-32 are also reported to target *PCAF*, a gene involved in p53 regulation and impacts tumour growth.[27] Following analysis of 365 miRNA and gene expression profiling in 60 newly diagnosed MM patients, significant deregulation of miRNA was noted in different cytogenetic subtypes.[28] For example, miR-203 and miR-342, located at 14q32, were downregulated in MM cells with t(4;14), and miR-1 and miR-113a were upregulated in MM cells with t(14;16).[28]

Centrosomes

The function of the centrosome is to direct the mitotic bipolar spindles for accurate chromosome segregation during mitosis.[29] Centrosome abnormalities in cancer correlate with chromosome instability and contribute to cell cycle regulation and checkpoints. Chromosomal instability and supernumerary centrosomes are typical in MM, representing early pathogenic events.

Single Nucleotide Polymorphisms

In MM and B cell development, highly polymorphic cytokine-encoding genes play an important role. When considering immune-related genes, an increased risk of MM was found to be associated with single polynucleotide polymorphisms (SNP) of *IL6R*, *IL1A*, *IL1RN*, *IL4R*, and *FCGR2A*,[30] whereas SNP in *IκBα*, an inhibitor of the NF-κB pathway and the transcriptional activator TRAF3, showed protective roles.[31] Other gene polymorphisms, including those in *IGF-1*, *IGFBP3*, and *IRS1*, related to insulin metabolism, also influence MM risk. Furthermore, polymorphisms in *SERPINE I*, *JAK3*, *CD4*, *RIPK1*, and *HPSE* immune-related and adhesion/growth genes have been associated with MM risk.[32] SNP in genes related to DNA repair, including *XRCC4*, *XRCC5*, and *ERCC2*, showed controversial effects on MM risk.[30]

MICROENVIRONMENT OF MULTIPLE MYELOMA

Bone Marrow Stromal Cells, Cytokines, and Endothelial Cells

The BM microenvironment plays a critical role in the pathogenesis of MM. The close interplay between MM cells and the microenvironment, including BM stromal cells (BMSC), vascular endothelial cells, osteoclasts and osteoblasts, fibroblasts, and adipocytes, modulates and sustains growth, survival, and the development of drug resistance of MM cells. Several genes of BMSC, which have major functions in RNA-processing (44 genes), cell cycle regulation (53 genes), and ubiquitin proteasome pathway activation (35 genes), are dysregulated when cocultured with myeloma cell lines.[33]

Table 1: Potential multiple myeloma treatments based on genetic mechanisms that promote disease progression.

	Genes	Cytokines/proteins	Functional site/pathway	Potential treatments (other than standard therapeutics)
Myeloma cells				Antibody-related therapies
Hyperdiploid	*CKS1B*, *ANP32E*, *Bcl9*, *PDZK1*, *NIK*, *TAC1*, *LTBR*	N/A	NF-κB	N/A
Non-hyperdiploid	*CCND1* (t[11;14]), *CCND3* (9t[6;14]), *MAF* (t[14;16])	N/A	G1/S phase	N/A
	FGFR3/MMSET (t[4;14]), *FAM46C*, *DIS3*, *CYLD* (16q), *BIRC2*, *BIRC3* (11q), *TRAF3* (14q32), *NIK*, *CD40*, *CDKN2C* (1q32)	N/A	NF-κB	Bortezomib MMSET inhibitors, FGFR3 inhibitors, and MEK inhibitors (t[14;16] or t[14;20]; e.g., cobimetinib)
		LTB		
	NRAS, *KRAS*, *BRAF*	N/A	MAPK/ERK	BRAF inhibitors (e.g., vemurafenib)
	TP53	N/A	PI3K/AKT	N/A
	Bcl2l1, *Mcl1*	N/A	IL6/STAT3	N/A
	sXBP1, *SP100*, *ROBO1*, *EGR1*	N/A	N/A	N/A
Epigenetics	*MMSET*	N/A	Histone modification	MMSET inhibitors
		BET		
microRNA	Upregulation: miR-let-7a, miR-16, miR-17-5p, mR-19b, miR-21, miR-531, miR-335, miR-342-3p, miR-25, miR-32, miR-20a, miR-93, miR-106a, miR-106b, miR-181a, miR-19b, miR-181b, miR-92a, miR-17-92. Downregulation: miR-372, miR-143, miR-155.	N/A	BCL-2, SOCS-1, IL6/STAT3, p53 regulation	miRNA mimics or inhibitors
Centrosome	N/A	N/A	Chromosome instability, cell cycle regulation and checkpoints	Chemotherapy, radiotherapy
Single nucleotide polymorphisms	*IL6R*, *IL1A*, *IL1RN*, *IL4R*, *FCGR2A*, *IκBα*	N/A	NF-κB	N/A
	IGF1, *IGFBP3*, *IRS1*	N/A	Insulin metabolism	N/A
	SERPINE I, *JAK3*, *CD4*, *RIPK1*, *HPSE*	N/A	Immunity or adhesion/growth	N/A
	XRCC4, *XRCC5*, *ERCC2*	N/A	DNA repair	N/A
	ALDH2, *GSTT2*, *BRCA1*, *SLC19A1*	N/A	N/A	Melphalan
	ABCB1, *CYP3A4*, *TP53BP2*, *GSTP1*, *TYMS*	N/A	N/A	Dexamethasone/ adriamycin/vincristine, and bortezomib
Microenvironment				
Stromal cells	44 genes	N/A	RNA processing	N/A
	53 genes	N/A	Cell cycle regulation	N/A
	35 genes	N/A	Ubiquitin proteasome pathway	N/A
	N/A	ICAM1/VCAM1	IL6/VEGF	N/A
	WNT5A, *WNT5B*, *WNT7A*, *WNT16*	N/A	WNT pathway	N/A

Table 1 continued.

	Genes	Cytokines/proteins	Functional site/pathway	Potential treatments (other than standard therapeutics)
	APC, *RBX1*, *FBXW1*	N/A	β-catenin	N/A
	CDC42, *ROCK1*	N/A	Planar cell polarity pathways	N/A
Cytokines	N/A	IL6, IGF1, VEGF, TNFα, IL21	JAK/STAT3, PI3K/AKT	N/A
	N/A	VEGF, SDF-1α, TNFα, IL21, IL6, IGF1	RAF/MEK/ p42/p44MAPK-dependent pathway	N/A
	N/A	HIF-1α, VEGF-A, HGF, syndecan-1	Angiogenesis	N/A
Endothelial cells	*DIRAS3*, *SERPINF1*, *SRPX*, *BNIP3*, *IER3*, *SEPW1*	N/A	Over angiogenic	N/A
Osteoblasts and osteoclasts	N/A	IL-6 and osteopontin increased	Proliferation, angiogenesis	N/A
Bone marrow fibroblasts	N/A	Integrin α5β5, periostin, MMP2, PDGFβ, laminin α4, PAI-1, MMC2	MM cell proliferation, survival, and migration	N/A
Immune cells	N/A	N/A	N/A	Daratumumab, elotuzumab, and CAR T cell therapy (e.g., anti-BCMA)
T helper 17	N/A	IL6, IL10, IL17, TGFβ	N/A	N/A
Regulatory T cells	N/A	IL10, TGFβ	Immunosuppression	N/A
Cytotoxic T cells	N/A	PD1 increased		Anti-PD1, e.g., pembrolizumab, nivolumab, and anti-PD-L1 (e.g., atezolizumab, avelumab)
Natural killer cells	N/A	PD1 increased, NKG2D decreased		
Tumour-associated macrophages	N/A	IL6, IL10, VEGFA, and nitric oxide increased	N/A	N/A

BCMA: B cell maturation antigen; CAR: chimeric antigen receptors; CD: cluster of differentiation; IL: interleukin; miRNA: microRNA; MM: multiple myeloma; N/A: not applicable; NF-κB: nuclear factor κ B; t: translocation; TGFβ: transcription growth factor β; TNFα: tumour necrosis factor α; VEGF-A: vascular endothelial growth factor A.

The VLA-4 on MM cells binds to fibronectin in the serum, and the LFA-1 on MM cells binds to ICAM1 on BMSC,[34] attracting MM cells to the BM. Other cytokines, such as TNF-α in the BM, can modulate the adhesion of MM cells by inducing NF-κB signalling. NF-κB-dependent upregulation of cell-surface adhesion molecules, such as ICAM1 and VCAM1, on both MM cells and BMSC, increases the binding capacity of tumour cells and BMSC, and induces the transcription and secretion of cytokines, such as IL-6 and vascular endothelial growth factor (VEGF) in BMSC.[31] Cytokines in the BM microenvironment, such as IL-6, insulin-like growth factor 1 (IGF-1), VEGF, and TNF-α, mediate the growth of MM cells. IL-6, IGF-1, and IL-21 are associated with tumour-cell survival and resistance to apoptosis,[35,36] which is mediated through the JAK/STAT3 and PI3K/AKT pathways. Other genes, including *ANXA2P1*

and *ANXA2P2*, are also related to myeloma cell adhesion and growth.[37]

The proliferation of MM cells is triggered by cytokines, such as IL-6, IGF-1, VEGF, TNF-α, stromal cell-derived factor-1α (SDF-1α), and IL-21, and is mediated through the RAF/MEK/p42/p44/MAPK signalling cascades.[35,36] NRG3, expressed exclusively in myeloma cells and MM BMSC, is able to activate ERBB4 and promote myeloma proliferation.[33] VEGF and SDF-1α, mediated through a protein kinase C and p42/p44/MAPK-dependent pathway, play important roles in cell migration. Wnt signalling, which is important in stemness, cell differentiation, and cell metabolism, is also deregulated in BMSC in MM patients. Upregulated expression of several non-canonical Wnt ligands (WNT5A, WNT5B, WNT7A, and WNT16), and upregulated expression of negative regulators of β-catenin (APC, RBX1 and FBXW1), would promote β-catenin ubiquitination for proteasome degradation.[33] Conversely, the upregulated expression of some members of the non-canonical Wnt/Ca^{2+} and planar cell polarity pathways (e.g., CDC42 and ROCK1) may indicate a possible enhancement of the migration and invasiveness properties in MM patients.[38]

Constitutive activation of HIF-1α and aberrant expression of HIF-2 by MM cells elevated levels of VEGF-A, hepatocyte growth factor, and syndecan-1, which are seen in the BM microenvironment and contribute to angiogenesis.[39] DIRAS3 (a GTP-binding RAS-like protein), SERPINF1, SRPX, BNIP3, IER3, and SEPW1 are correlated with the over-angiogenic phenotype of MM endothelial cells in active disease.[40]

Bone Marrow Niches

In addition to BMSC, osteoblastic and osteoclastic niches contribute to MM cell proliferation and angiogenesis through secretion of IL-6 and osteopontin.[16]

BM fibroblasts from MM produce ECM proteins, including integrin α5β5, periostin, MMP2, PDGF β, laminin α4, plasminogen activator inhibitor-1, lysyl-hydroxylase 2, prolyl 4-hydroxylase 1, nidogen-2, c-type mannose receptor-2, and basigin.[41] Both BM fibroblasts and adipocytes are shown to support MM cell proliferation, survival, and migration.

Immune Cells

Immune cells show important supportive roles in MM. T helper 17 cells are abundant in the BM, under the priming of elevated concentrations of IL-6 and transcription growth factor-β, which suppress cancer immune surveillance by secreting IL-17 and IL-10. Increased proportion of functional regulatory T cells in the peripheral blood of MM patients directly correlates with worse prognosis.[42] Cytotoxic T cells showed increasing expression of PD-1, a T cell receptor coreceptor with inhibitory function, in MM BM.[43] Natural killer (NK) cells are also functionally impaired in MM patients with downregulation of the NK group 2D activating receptor and upregulation of inhibitory coreceptor PD-1. Type I NK T cells, expressing an invariant TCR, have been reported to play an important role in anticancer surveillance, and Type I NK-T deficiency was associated with MM progression and relapse.[44] Tumour-associated macrophages contribute to MM pathogenesis in three ways: as a major source of IL-6; by producing IL-10, a major mediator of cancer immune tolerance by suppressing the function of T cells; and by releasing VEGF-A and nitric oxide.[45]

RISK STRATIFICATIONS

Risk category is based on the biological and molecular profile that predicts prognosis and treatment responses. Hyperdiploid myeloma and t(11;14) confer relatively favourable prognoses. *MAF* (t[14;16]), *MAFB*, or *FGFR3/MMSET* (t[4;14]) activation and deletion on chromosome 13 and/or 17 are associated with poor prognosis.[6,46] GEP70 is used to identify high-risk disease and 10 subgroups are mentioned.[3] Revised International Staging System (ISS) analysed 3,060 newly diagnosed MM patients.[46] Based on ISS stages (β2-microglobulin and albumin), chromosome abnormalities (CA) and lactate dehydrogenase levels, the risk was categorised into three groups, from low-risk revised-ISS group I with ISS Stage I; no high-risk CA (del[17p] and/or t[4;14 and/or 14;16]) and normal LDH level; to high-risk R-ISS group III with ISS Stage III and high-risk CA or high LDH level.[46]

Mayo Stratification for Myeloma and Risk-Adapted Therapy divided active MM into

three groups: high-risk, including del(17p), t(14;16), t(14;20), and GEP high-risk signature; intermediate risk, including t(4;14), del(13), hypodiploidy, and PC labelling index ≥3%; and standard risk, including t(11;14), and t(6;14). Patients with t(4;14) were shown to be at an advantage with bortezomib treatment.[47]

In cells with centrosome abnormalities, the threshold of apoptosis activation induced by drugs or radiation may be much lower than that in other cells.[29] This may be because the functions of centrosomes are upstream of mitochondrial proteins (such as cytochrome c), Bcl-2 family proteins, and other apoptosis molecules such as Blc homology domain 3-only proteins and caspase 8, Noxa, TR3, BAK and BID, and TP53, which play an important role in cell cycle regulation and apoptosis.

TREATMENT

Current Treatment

Based on patients' age, performance status, and other comorbidities, individuals with MM can be divided into autologous haematopoietic stem cell transplantation (ASCT)-eligible and ASCT-ineligible groups. Choosing the optimal initial therapy in MM remains a challenge. There are currently at least five classes of active agents available for the treatment of myeloma: alkylating agents (melphalan and cyclophosphamide), anthracyclines (adriamycin®, Pfizer, New York, New York, USA, and liposomal doxorubicin), corticosteroids (dexamethasone and prednisone), immunomodulatory drugs (thalidomide and lenalidomide), and proteasome inhibitors (bortezomib, carfilzomib, and ixazomib). Mayo Stratification for Myeloma and Risk-Adapted Therapy guidelines[47] suggest four cycles of bortezomib, lenalidomide, and dexamethasone before ASCT, followed by ASCT and lenalidomide maintenance therapy, or, alternatively, tandem ASCT and bortezomib-based maintenance therapy, in standard and intermediate-risk patients, respectively. For high-risk patients, four cycles of carfilzomib, lenalidomide, and dexamethasone therapy, followed by ASCT or tandem ASCT and carfilzomib or bortezomib-based maintenance therapy for 2 years, was recommended. For ASCT-ineligible patients, bortezomib, lenalidomide, and dexamethasone therapy is suggested for all risk groups for 1 year and followed by lenalidomide and dexamethasone therapy in standard risk patients, and bortezomib-based maintenance therapy for a minimum of 1 year for intermediate or high-risk patients. In standard-risk patients >75 years old, lenalidomide and dexamethasone therapy was suggested as an initial treatment. However, this guideline has never been tested in prospective clinical studies to show superiority over other non-preferred regimens.

Molecular-Based Therapy

MM cells characterised by gene alteration involved in TNF/NF-kB signalling and antiapoptosis showed better responses and longer progression free survival (PFS) with bortezomib use.[48] MM patients with t(4;14) (overexpression of MMSET and FGFR3) have better overall survival (OS) with bortezomib treatment.[49] MMSET, FGFR3, and MEK inhibitors could be potential treatment choices in patients with t(14;16) or t(14;20). *BRAF* Val600Glu mutation, accounting for 4% of MM patients, is the target of *BRAF* inhibitors.[49] To treat patients with unfavourable GEP, novel inhibitors (e.g., AURKA inhibitors) target overexpressed genes.[49]

SNP in MM cells also contribute to treatment responses. The *TNFα-238A* allele was correlated with prolonged PFS and OS in patients treated with thalidomide and dexamethasone.[50] Treatment response of melphalan was associated with SNP in *GSTP1*, *ALDH2*, *GSTT2*, *BRCA1*, and folate transporter *SLC19A1*. Polymorphisms in *ALDH2* and *CYP1A1* were correlated with prolonged OS.[51] Treatment response of dexamethasone/adriamycin/vincristine was correlated with SNP in *ABCB1*, *CYP3A4*, *TP53BP2*, *GSTP1*, and thymidylate synthase.[52] T allele polymorphism in *ABCB1* has been shown to have a better response to dexamethasone/adriamycin/vincristine and bortezomib treatment, as well as better PFS and OS.[53] The poor metaboliser phenotype of *CYP2C19* was associated with a poor response to thalidomide and increased risk of peripheral neuropathy.[54]

Other therapeutic approaches have been demonstrated in preclinical studies by

investigating the activity of miRNA mimics or inhibitors on tumour cells. Certain miRNA showed increasing drug sensitivity, including LNA-i-miR-221 to melphalan, miR-2012 mimics to bortezomib, miR-150-5p mimics to glucocorticoids, and miR-29b to bortezomib, carfilzomib, and ixazomib.[55]

Antibody-Related Therapies

Several antigens that exhibit strong expression in MM cells, including cluster of differentiation (CD) 38, CD138, CD56, CD74, CD40, IGF-1R, SLAMF7, and immunoglobulin superfamily member FcRL5, may be candidates for antibody-related immunotherapy.[56] Daratumumab and SAR650984 are anti-CD38 monoclonal antibodies that have shown satisfactory response rates in patients with relapsed/refractory MM and CD38+ haematological malignancies in separate Phase I clinical trials. A Phase III clinical trial of daratumumab, bortezomib, and dexamethasone, compared with bortezomib and dexamethasone, showed better overall response rate (ORR) (82.9% versus 63.2%) and better 1-year PFS (60.7% versus 26.9%) in 498 relapse/refractory MM cases.[57] However, daratumumab monotherapy showed a reduced effect with an ORR of 31.1% in relapse/refractory MM.[58] This reduced ORR may be a result of the enrolled patients having a higher refractory rate to last line of therapy compared to previous studies (62.2% versus 30.3%), which may reflect higher clonal heterogeneity, and missing stimulatory component of immunomodulatory drugs (IMiD) and bortezomib.

Elotuzumab is an antisignalling lymphocyte activating-molecule F7 monoclonal antibody. The mechanism of action of elotuzumab includes mediating antibody-dependent cell-mediated cytotoxicity, enhancing NK cell cytotoxicity, and disrupting MM cell adhesion to BMSC.[59] The combination of elotuzumab, lenalidomide, and dexamethasone yielded an ORR of 79% in patients with refractory/relapsed MM in a Phase III ELOQUENT-2 clinical trial.[60] There are two Phase III studies in newly diagnosed MM patients: ELOQUENT-1[61] and GMMG HD6.[62] In ELOQUENT-1, 375 patients received elotuzumab plus lenalidomide and dexamethasone, and 375 patients received the control treatment of lenalidomide and dexamethasone; the results of this trial have not yet been published. In the GMMG HD6 Phase III clinical trial, 516 newly diagnosed MM patients are to be enrolled, and it aims to compare bortezomib, lenalidomide, and dexamethasone, with or without elotuzumab, in consolidation and maintenance therapies; this trial is also ongoing.

Other potential monoclonal antibodies include milatuzumab (an anti-CD74 monoclonal antibody),[63] dacetuzumab,[64] and lucatumumab.[65] The anti-CD40 monoclonal antibodies, siltuximab, targeting IL-6,[66] showed partial response (PR) in refractory/relapse MM. All of the aforementioned monoclonal antibodies should be considered for further personal treatment.

Another type of antibody-related therapy is antibody-drug-conjugated therapy: e.g., indatuximab ravtansine (BT062), an anti-CD138 antibody-drug conjugate (ADC);[67] lorvotuzumab mertansine, an anti-CD56 ADC;[68] and milatuzumab, an anti-CD74 ADC with doxorubicin,[63] which induced a response in relapse/refractory MM as a monotherapy or combined with other drugs. The anti-FcRL5 maytansine analogue, DM4, and monomethyl auristatin E;[69] the anti-B cell maturation antigen (BCMA), C269, antibody conjugated to monomethyl auristatin F;[70] and the anti-BCMA ADC GSK2857916[71] showed good response in preclinical studies.

Immunotherapy

IMiD, including thalidomide, lenalidomide, and pomalidomide, induce an immune response. The drugs inhibit TNF-α production and angiogenesis by blocking the angiogenic growth factors, including basic fibroblast growth factor and VEGF. Specifically, these agents trigger and enhance caspase-8-mediated MM cell apoptosis and enhance both caspase-8-mediated MM cell apoptosis, triggered by FAS or TRAIL, and caspase-9-mediated MM cell killing, triggered by dexamethasone. IMiD also block the induction of cytokines, such as IGF-1, and inhibit IL-6 and VEGF secretion triggered by MM cell adherence to BMSC. In addition, IMiD inhibit angiogenesis and augment NK cell activity against autologous MM cells.[72] Several clinical trials have demonstrated the benefits of using regimens involving thalidomide or IMiD, including lenalidomide and pomalidomide, for MM treatment, particularly in combination

with proteasome inhibitors.[73-76] This combined therapy has become the standard regimen for MM treatment.

T cells redirected to specific antigen targets with engineered chimeric antigen receptors (CAR) are emerging as powerful therapies in haematologic malignancies. Garfall et al.[77] reported a refractory MM patient who achieved complete response (CR) while receiving autologous transplantation followed by treatment with CTL019 cells, which consists of autologous T cells expressing a CD3-ζ/CD137-based anti-CD19 CAR from a lentiviral vector. BCMA, a member of the TNF receptor superfamily TNFRSF17, is virtually absent on naïve and memory B cells, but is selectively induced during PC differentiation where it supports humoral immunity by promoting the survival of normal PC and plasmablasts. Ali et al.[78] reported the first in-human clinical trial of T cells expressing anti-BCMA CAR in 12 advanced, heavily pretreated MM patients. Among these patients, one achieved stringent CR, one achieved very good partial response (VGPR), two achieved PR, and 8 patients had stable disease.[78]

The immune checkpoint inhibitor, PD-1, is upregulated on the surface of activated T cells, and its ligands (PD-L1 and PD-L2) are expressed on the surface of antigen-presenting cells and tumour cells. Pembrolizumab is a monoclonal antibody against PD-1 that helps to restore antitumour immune surveillance. KEYNOTE-023 is a Phase I dose-escalation study evaluating the safety and efficacy of pembrolizumab in combination with lenalidomide and low-dose dexamethasone in patients with relapse/refractory MM.[79] With a median follow-up of 9.7 months (range: 4.3–18.4), 76% (13/17) of the patients evaluated for efficacy in dose determination/confirmation responded to treatment, including 4 VGPR and 9 PR, with a median duration of response 9.7 months (0.0–16.7). Three patients (18%) had stable disease. M protein or free light chains were reduced in 94% of patients.[80] Badros et al.[81] reported a Phase II study of 48 relapsed/refractory MM patients with at least two prior lines of therapy, treated with pembrolizumab, pomalidomide, and dexamethasone. ORR of PR or better was observed in of 27 of 48 patients (56%): stringent CR (8%), normal CR (6%), VGPR (13%), and PR (29%). Of the 18 high-risk patients, ORR was 33% including VGPR (11%) and PR (22%); however, another anti-PD1 antibody, nivolumab, did not show objective responses in MM.[82] This may be attributed to the fact that the mechanism of action of T cell activity against MM cells does not involve PD-1 interaction with PD-L1.

Unlike ASCT, allogeneic stem cell transplant is a potentially curative option in MM, especially for the high-risk subgroup, which has several advantages, including a tumour-free graft and the potential for sustained immune-mediated disease control. However, the role of allogeneic stem cell transplant is limited due to high treatment-related mortality with conventional myeloablative conditioning regimens and controversial benefit in reduced-intensity/non-myeloablative conditioning regimens.[83] Vaccination with dendritic cell and tumour fusions following ASCT reported marked expansion of myeloma-specific T cells and cytoreduction of minimal residual disease.[83] In a Phase II clinical trial, Rosenblatt et al.[84] demonstrate that repeated immunisation with a dendritic cell and tumour fusion vaccine after ASCT improved clinical response and late response rate after ASCT. Lenalidomide was reported to promote T cell proliferation and augment response to myeloma-specific tumour vaccines.[85]

CONCLUSIONS

Because of heterogeneities and clonal evolution of MM, a full understanding of the genetics of myeloma and its integration with standard clinical prognostic information may help design specific trials and treatments, especially for high-risk patients. Molecular-based therapies targeting MM cells and microenvironment have been studied recently. Immunotherapies, including CAR-T and checkpoint inhibitors, have shown promising results in relapse and refractory MM, with some effects in high-risk patients. However, a real, personalised, approach is still far behind the medical community, as CAR-T approaches with current antigenic selections and the current constructs have not really produced reliable effects. In the future, drugs targeting molecular pathways and immune therapies will be important for personalised treatment.

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Modern Methods for Studying Portal Hypertension-Associated Angiogenesis in Experimental Research

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Abstract

Portal hypertension (PH) is associated with the majority of severe complications occurring in liver cirrhosis, and an increase in hepatic vascular resistance to portal blood flow underlies its pathogenesis. The reasons for this include gross structural rearrangement of the liver due to diffuse fibrosis and regenerative nodule formation, remodelling, and capillarisation of sinusoids, as well as endothelial dysfunction and impaired paracrine interaction between damaged hepatocytes, sinusoidal endothelial cells, Kupffer cells, and activated hepatic stellate cells. In this situation, intrahepatic angiogenesis is considered a compensatory mechanism aimed at portal system decompression. At the same time, newly formed vessels bypass sinusoids, so they are unable to provide oxygen and nutrients to the liver tissue, which causes disease deterioration. Angiogenesis, vascular remodelling, and endothelial dysfunction result in portosystemic shunt formation, splanchnic congestion, and hyperdynamic circulation, which leads to PH progression and the occurrence of inherent complications, in particular bleeding from oesophageal varices. Given the significant role of vascular formation and remodelling in the development of PH in cirrhosis, studying angiogenesis is important for the development of antiangiogenic therapy, which could improve associated haemodynamic disorders. To this end, various experimental models and methods, both *in vitro* and *in vivo*, of qualitative and quantitative evaluation are used.

INTRODUCTION

Portal hypertension (PH) is associated with the majority of severe complications occurring in liver cirrhosis, and an increase in hepatic vascular resistance to portal blood flow underlies its pathogenesis. The reasons for this include gross structural rearrangement of the liver due to diffuse fibrosis and regenerative nodule formation, remodelling, and capillarisation of sinusoids, as well as endothelial dysfunction and impaired paracrine interaction between damaged hepatocytes, sinusoidal endothelial cells, Kupffer cells, and activated hepatic stellate cells. In this situation, intrahepatic angiogenesis is considered a compensatory mechanism aimed at portal system decompression. At the same time, newly formed vessels bypass sinusoids, so they are unable to provide oxygen and nutrients to the liver tissue, which causes disease deterioration.[1] Angiogenesis, vascular remodelling, and endothelial dysfunction result in portosystemic shunt formation, splanchnic congestion, and hyperdynamic circulation, leading to PH progression and the occurrence of inherent complications, in particular bleeding from oesophageal varices.[2] Given the significant role of vascular formation and remodelling in the development of PH in cirrhosis, studying angiogenesis is important for the development of antiangiogenic therapy, which could improve associated haemodynamic disorders.[3] To this end, various experimental models and methods, both *in vitro* and *in vivo*, of qualitative and quantitative evaluation are used.[4]

INTRAHEPATIC ANGIOGENESIS ASSAYS

Appearing simultaneously with fibrosis, intrahepatic angiogenesis causes abnormal angioarchitecture formation characteristic of cirrhosis, which is the endpoint of the disease. Therefore, evaluation of intrahepatic angiogenesis is necessary to assess disease progression and search for therapeutic targets (Table 1).[5]

Scanning Electron Microscopy

Scanning electron microscopy is the traditional method for studying the three-dimensional (3D) structure of microcirculation. It is visualised by an electron beam after intravascular injection of coloured gelatin, latex, or plastic casting material followed by tissue clearing or corrosion. Unlike tissue sections, this approach makes it possible to not only quantify vessel dimensions, intervascular distances, branching order, and luminal surface features, but also to mathematically calculate the wall shear stress.[6] Using scanning electron microscopy in rats with cirrhosis caused by subcutaneous injections of carbon tetrachloride (CCl_4) (0.3 mL 50% CCl_4 diluted with oil per 100 g of body weight twice a week for 3 months), it was determined that the number of sinusoidal endothelial fenestrae decreased and the sinusoids within the regenerative nodules surrounded by fibrous septa were narrow.[7] In mice with biliary cirrhosis, this technique allowed for the identification of numerous blindly terminating and chaotically located sinusoids, as well as large portosystemic collaterals bypassing them and shunting blood towards the hepatic veins. The disadvantage of bile duct ligation is biloma formation, causing irregular saccular deformation of sinusoids, which presents as holes in the cast. Moreover, limitations of the casting technique include perfusion difficulties, especially for microvascular perfusion, and bloating of the sinusoids due to the injection pressure. This makes it difficult to perform a more thorough morphometric analysis of the hepatic microvasculature.[8]

Intravital Fluorescence Microscopy

Intravital fluorescence microscopy is necessary for the intravital evaluation of structural changes at the microcirculatory level in experimental cirrhosis. For this purpose, different models of cirrhosis may be used, such as bile duct ligation and subcutaneous or intraperitoneal administration of CCl_4.[9] After performing midline and subcostal incisions, the hepatic ligaments were dissected, and the left liver lobe was placed on a fixed plate to minimise respiratory movements. A fluorescent dye was injected into the jugular or tail vein, and, while tissue contrast is increased, liver microcirculation was analysed with a fluorescent microscope.

Intravital fluorescence microscopy makes it possible to visualise hepatic stellate cells due to autofluorescence of vitamin A; estimate the number of rolling and adherent leukocytes; and measure relative vascular density, the diameter and perfusion of sinusoids, and flow and volume velocity of blood.[10,11]

Table 1: Advantages and disadvantages of intrahepatic angiogenesis assays.

Assay	Advantages	Disadvantages
Scanning electron microscopy.	Quantification of vessel dimensions, intervascular distances, branching order, and luminal surface features, as well as the wall shear stress.	Perfusion difficulties of casting materials, especially for microvascular perfusion. Biloma formation after bile duct ligation causes holes in the cast.
Intravital fluorescence microscopy.	Evaluation of microcirculatory structural changes *in vivo*; hepatic stellate cells visualisation; and the possibility to estimate the number of rolling and adherent leukocytes, relative vascular density, the diameter and perfusion of sinusoids, and flow and volume velocity of blood.	Visualisation of only superficial structures and the technique is not capable of estimating the diameter of portal venules. Hard to perform if intraperitoneal injections of CCl_4 or bile duct ligation are used.
Three-dimensional microcomputed tomography *in vivo*.	May be repeatedly conducted, thereby allowing gradual monitoring. Three-dimensional images of vasculature.	Resolution is relatively low; therefore, it is not possible to evaluate microcirculation. Perfusion difficulties of casting materials.
Three-dimensional microcomputed tomography *ex vivo*.	Higher resolution, compared to *in vivo* microCT; allows analysis of the microcirculation, as well as large vessels; and gives three-dimensional images of vasculature.	Perfusion difficulties of casting materials.
Immunohistochemical methods.	Allows the evaluation of newly formed vessels without a lumen and of proangiogenic factors expression.	Analysis of microcirculation only.
Confocal laser scanning microscopy after immunohistochemical staining.	Three-dimensional images of vasculature, provides a high-resolution image and volumetric data on microcirculation.	Tissue shrinkage and deformation hampers confocal microscopy. Limited diffusion of (primary) antibody penetration.

The maximal number and diameters of portosystemic shunts were observed 3 weeks after bile duct ligation and 12 weeks after the first administration of CCl_4 (0.1 mL 50% oil solution per 100 g of body weight subcutaneously twice a week for 4 months; 5% alcohol was added to drinking water).[10] Fibrosis and the activated hepatic stellate cells were located in periportal areas in biliary cirrhosis and in pericentral areas in CCl_4-induced cirrhosis. The sinusoids in these regions became narrow, whereas the distance between them increased.[10]

Although subcutaneous CCl_4 administration can induce necrosis at the site of injection and should be carried out for 16 weeks, this route of administration is preferable for intravital fluorescence microscopy.[10,12] In contrast to intraperitoneal injections or bile duct ligation, subcutaneous CCl_4 administration does not cause adhesions between the liver and the neighbouring organs that can later limit the possibility of carrying out *in vivo* studies in the abdominal cavity. Despite the fact that the oral application of CCl_4 enhances mortality rate,[13] a modification of this technique by Proctor and Chatamra[14] has been developed. The dose was individualised according to weight gain/loss of the animal in response to the previous dose. Short inhalation cycles of CCl_4 two or three times weekly should also be considered to avoid extrahepatic damage.[15]

The major drawback of intravital fluorescence microscopy is that it can only visualise superficial structures; therefore, it does not give an opportunity to estimate the diameter of portal venules. This may be due to the displacement of portal tracts deeper into the tissue as a result of pericentral fibrosis.

Three-Dimensional Microcomputed Tomography

Microcomputed tomography (microCT) provides high-resolution 3D images that are composed of two-dimensional (2D) trans-axial projections, or 'slices', of a target object. For live animal

imaging, the slices are obtained by rotating the emitter and detector. To evaluate microcirculation, *ex vivo* microCT requires vascular casting with contrast agents, such as microfil MV-122 and $BaSO_4$/gelatin, whereas *in vivo* microCT can be performed with iodinated monomer-based bolus or lipid emulsion-based blood-pool contrast agents.[16]

Intravital microCT is performed with a dual-energy flat-panel microCT scanner before and immediately after intravenous injection of 100 µl of specially optimised iodine-based contrast agent eXIA™160XL (Binitio Biomedical Inc., Ottawa, Canada). A Feldkamp-type algorithm is used to reconstruct 2D images into 3D with a voxel size of 35x35x35 µm. The relative blood volume value determination is based on the mean brightness of the liver tissue after a contrast agent injection. The value correlates with the number of angiogenic vessels. In this way, a statistically significant increase in the relative blood volume was observed in the murine cirrhotic liver 6 weeks after the first intraperitoneal administration of CCl_4 (0.06 mL 50% oil solution per 100 g of body weight twice a week for 6 weeks) and 2 weeks after bile duct ligation. The advantage of the technique is that it can be conducted repeatedly, thereby allowing gradual monitoring of the process.

The merit of *ex vivo* microCT is the higher resolution image obtained.[17,18] It was used in different models of cirrhosis in mice and rats. In its classical version, the inferior vena cava is crossed above the diaphragm, and the radiopaque lead oxide diluted in a liquid silicone polymer (microfil) was injected into the portal vein at the rate of 8–10 mL/min and at a pressure of 10–12 mmHg; or alternatively, injection into the heart at a pressure not higher than that of the artery, this is carried out using an automatic pump. The specimens are kept at 4°C for 12 hours. Subsequently, the liver is taken, cut into lobes, fixed in formalin, and then dehydrated in increasing concentrations of a glycerol aqueous solution at 24-hour intervals. Using a microCT scanner and the special computer processing algorithm, 3D images of the intrahepatic microvasculature are obtained and then analysed using software.[19]

MicroCT *ex vivo* makes it possible to estimate the ratio of vascular volume to total liver volume and enables precise analysis of the branching of medium and large hepatic vessels. They are detected in the liver, particularly at the periphery, 6 weeks after the first intraperitoneal injection of CCl_4 (0.06 mL 50% oil solution per 100 g of body weight twice a week for 6 weeks) and 21 days after bile duct ligation. By Week 4 of biliary cirrhosis development, the vascular volume increased one and a half times and the number of branches doubled,[20] which reflected the severity of angiogenesis.[21]

The limitations of the technique include perfusion difficulties, especially for microcirculatory perfusion, and the reactivity of casting resins with other chemical compounds and surrounding tissue. Moreover, dual casting, which is necessary for contrast-based differentiation between venous and arterial systems, is not possible. This is due to the presence of shunts between the hepatic arterioles and portal venules, functioning as a one-way valve that allows blood to flow only from the arterial to venous system.[22] To overcome this shortcoming, Peeters et al.[23] sequentially injected yellow or blue contrast agents, PU4ii, into the abdominal aorta or portal vein, respectively, after clamping the thoracic aorta and renal arteries. In order to prevent damage to the microvessels by the pumped substance, a polyethylene drainage tube was installed into the inferior vena cava through the right atrium. The thoracic section of the inferior vena cava, the abdominal aorta, and the portal vein were clamped to eliminate leakage of the substance during polymerisation. After 72 hours, the casted liver was macerated using a 25% potassium hydroxide bath for 5 days. The vascular replica was then flushed with distilled water and laid to dry under a vented hood for a further 5 days. MicroCT with 3D reconstruction was performed with a resolution of 1.89 µm for microcirculation and 40 µm for larger vessels. To morphologically analyse microcirculation, a sample with the dimensions 350x350x200 µm was virtually dissected in between portal triads. The average radius of the sinusoidal vessels, branch length, tortuosity, and porosity (the total sinusoidal volume divided by the volume of its envelope) of the vascular network was assessed with the in-house developed software. Using this technique in animals with macronodular cirrhosis induced by thioacetamide (first, 0.03% thioacetamide was added to drinking water, and then the concentration was adapted every

week for 18 weeks to keep body weight within a 250–300 g range), these authors have identified the compression of the hepatic venules and an increase in the diameter of the hepatic artery.[24]

Immunohistochemical Methods

Intravascular injection of contrast agents only revealed the structure of functioning vessels. Immunohistochemical staining of tissue sections is used for a more accurate evaluation of the newly formed vessels, including the nascent capillaries without a lumen. The most common specific markers are vascular endothelial growth factor (VEGF) and membrane proteins CD31 and CD34 for endothelial cell detection. In particular, VEGF and angiopoietin-1 expression shows an increase in intrahepatic vascular density in rats with biliary cirrhosis.[21] Immunofluorescence is a variant of immunohistochemical staining. It requires the use of secondary antibodies such as streptavidin conjugated with carbocyanine CY2.[25]

Confocal laser scanning microscopy after immunohistochemical staining makes it possible to study 3D structures with a resolution of up to 0.2 µm. This technique is based on improved protocols for the chemical purification of samples allowing a dye and photons to penetrate deeper into tissue before and after immunohistochemistry.[26,27] Subsequent confocal laser scanning provides detailed volumetric data on microcirculation at a voxel size of 0.63x0.63x1.40 µm. The data are processed by a specially developed software (DeLiver). In a study involving rats with thioacetamide-induced cirrhosis, a decrease in mean radius and porosity as well as an increase in tortuosity and length of sinusoids were determined using this technique.[24]

EXTRAHEPATIC ANGIOGENESIS ASSAYS

Violations of organ and systemic haemodynamics and portosystemic collateral circulation in PH begin with splanchnic vasodilation and neovascularisation, which are caused by hypoxia of the small intestinal mucosa with the participation of proinflammatory cytokines, chemokines, and angiogenic factors such as VEGF, platelet-derived growth factor, phosphatidylinositol glycan anchor biosynthesis class F, and others. Portosystemic shunts become clinically significant when in the presence of gastro-oesophageal varices, the rupture of which leads to life-threatening bleeding. It was traditionally thought that these varices are formed when increased portal pressure causes the opening of pre-existing vessels in the areas of embryonic communication between the portal and systemic circulation. This paradigm was challenged by Fernandez et al.,[28] who first reported that portosystemic collaterals in PH are formed due to active angiogenesis (Table 2).

Intravital Microscopy of The Small Bowel Mesentery

Intravital microscopy enables imaging of structural changes of microvasculature, vascular permeability, and mesenteric vascular density, which characterises splanchnic angiogenesis. After performing a midline laparotomy, a small intestinal loop was exteriorised, placed on a heated plexiglas plate, and continuously superfused with an Earle's balanced salt solution to prevent dehydration. Observations were carried out with the Axiotech Vario 100HD microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with water immersion objectives (x10 and x40). The obtained image was recorded for the subsequent computer analysis. This enabled study of all the types of mesenteric microvessels and calculation of their density, which is defined as the ratio of a vessel's length to the area it occupies.[29] Epifluorescence microscopy is used to measure vascular permeability. After the selection of a venular segment with a diameter of 20–40 mm and an unbranched length of about 150 mm, fluorescent isothiocyanate-bovine serum albumin was injected intravenously. As the intraluminal grey scale value fell, the perivascular grey scale value rose when the fluorescent isothiocyanate-bovine serum albumin molecule leaked through the vascular wall, quantified using black and white image and taking black for 0 and white for 255.

Intravital microscopy revealed an increased vascular density and gross disturbance of the mesenteric microcirculation in rats with PH induced by partial portal vein ligation (PPVL) and in rats with biliary cirrhosis. Moreover, the changes in the latter were more significant, which may be due to the time required for cirrhosis development. In addition, vascular permeability was significantly increased in these

animals, in contrast to permeability in animals with extrahepatic PH. This is explained by the higher levels of endothelial nitric oxide synthases and VEGF.[30]

In addition to the aforementioned uses, intravital microscopy is used to quantify adhesion and rolling of leukocytes, a well-known hallmark of inflammation, which in turn leads to angiogenesis. In particular, in rats with cirrhosis induced by CCl_4 (0.04 mL administered intragastrically and increased weekly in increments of 0.04 mL to a maximum dose of 0.4 mL; 35 mg/100 mL phenobarbital was added to drinking water once 2 weeks before the first administration of CCl_4), the index of leukocyte-endothelial interaction was increased in the microcirculation of the liver and small bowel mesentery.[31] The disadvantage of this method is the need for surgical intervention and extraction of the mesentery from the abdominal cavity. This causes a rapid and pronounced increase in the number of leukocyte rolling in response to partial degranulation of perivascular mast cells and endothelium expression of P-selectin in a matter of minutes.[32]

The Requirements for the Analysis of Microcirculation Images Obtained with Intravital Microscopy

Intravital microscopy requires video recording for the analysis of microvasculature. It is recommended that the following requirements are met:[33]

- At least three, or preferably five, arbitrary regions of microcirculation should be included in the analysis.
- Optical magnification should be x10 for microcirculation imaging in small laboratory animals.
- It is necessary to avoid pressure artifacts occurring when a microscope objective contacts the region of interest; excess pressure applied to the area may collapse the microcirculation and stop venous blood flow.

Table 2: Advantages and disadvantages of extrahepatic angiogenesis and portosystemic shunting assays.

Assay	Advantages	Disadvantages
Intravital microscopy of the small bowel mesentery.	Enables imaging of structural changes of microvasculature, vascular permeability, adhesion and rolling of leukocytes, and mesenteric vascular density.	The need for surgical intervention, which limits repeated procedures because of adhesions formation. Rapid increase in the number of leukocyte rolling.
Teflon rings implantation.	Corresponds to an *in vivo* situation with an intact circulation, enables the evaluation of newly formed vessels without a lumen, and the evaluation of proangiogenic factors expression.	The need for surgical intervention.
Immunohistochemical methods.	Evaluation of newly formed vessels without a lumen and of proangiogenic factors expression.	Analysis of microcirculation only.
Scanning electron microscopy.	Quantification of vessel dimensions, intervascular distances, branching order and luminal surface features, as well as the wall shear stress.	Perfusion difficulties of casting materials, especially for microvascular perfusion.
Microsphere technique.	Evaluation of total shunting degree, as well as splenorenal shunting.	The need for laboratory animals.
Three-dimensional micro-single-photon emission computed tomography.	Serial measurements of portosystemic shunting Three-dimensional imaging *in vivo*. Pre-interventional measurement of portosystemic shunting that is important for selection of animals with similar baseline characteristics in studies evaluating antiangiogenic therapy.	Requires the use of radioactive material.

- Video images should be stored in full size without compression to the form of DV-AVI files in order to provide the possibility of computer frame-by-frame analysis. The optimal video recording time is 20 seconds.

A report on the analysis of images obtained with intravital microscopy must include the following parameters calculated for all vessels and capillaries separately:

- Total vascular density and perfused vessel density, which are calculated as the ratio of total vessel length to image area.
- Proportion of perfused vessels expressed as a percentage.
- Microvascular flow index.
- Heterogeneity index, which is calculated as the ratio of the difference between the maximum and minimum blood flow velocity to its average velocity in the five selected areas of an image.

Calculations can be made directly by the researcher using semiquantitative scales for visual evaluation.[34,35] For a more accurate analysis of the required parameters, the CapImage software is used. It was specially developed for intravital microscopy.[36]

In Vivo Evaluation of Angiogenesis in the Small Bowel Mesentery by Implantation of Teflon Rings

Implantation of Teflon rings is another technique for intravital evaluation of angiogenesis of the small bowel mesentery in PH. The rings have a diameter of 7 mm, a height of 3 mm, and an internal diameter of 5 mm. The rings are placed into polyester mesh bags and filled with a mixture of bovine Type I collagen and bovine serum albumin. After performing a midline incision and PPVL, the rings were implanted between the two mesenteric membranes and fixed with single sutures in rats. After 16 days, the rats were euthanised, the implant was removed, fixed in a 4% formaline, and paraffinised. Then, 3-µm-thick tissue sections were prepared. The tissue was stained for further video morphometry and vascular density calculation. This technique makes it possible to determine the number of vessels and the mechanisms of their formation.[37]

Immunofluorescence Assay

Vascular network imaging can be carried out by immunological reaction of fluorescent antibodies with membrane proteins of endothelial cells. Anti-CD31 and anti-VEGF antibodies are most often used for these purposes.[38,39] The small intestinal wall or its mesentery was washed in sodium phosphate buffer, dried on gelatin-coated slides, and fixed in 100% methanol at -20°C for 30 minutes. The sections were then incubated with the corresponding primary murine anti-rat antibodies at 4°C for 12 hours. Streptavidin conjugated with carbocyanine CY2 was used as a secondary antibody. It was applied at room temperature and held for 1 hour. The image obtained after fluorescence microscopy may be analysed using ImageJ software.[40] An alternative technique consists of the fixation of frozen sections in acetone at -20°C for 10 minutes. After which, the tissue is blocked with 5% bovine serum albumin solution for 45 minutes.[41] Besides CD31, endothelial cell identification is possible with the use of BSI-lectin. Perivascular cell markers include Neural/glial antigen 2, desmin, α-smooth muscle actin, platelet-derived growth factor receptor-β, and Class III β-tubulin.[42]

Immunohistochemical Staining

Immunohistochemical staining is performed to study not only intrahepatic but also extrahepatic angiogenesis, including angiogenesis in the small bowel mesentery and the gastric wall. The presence of angiogenesis in the small bowel mesentery was confirmed by numerous experimental studies in laboratory animals with different PH models.[43]

Tissue oxidative stress, which occurs in PH, aggravates the pathophysiological changes that occur in the gastric wall. The oxidative stress is detected by the reaction of antibodies with metabolites that arise during free radical oxidation, such as nitrotirozine. In particular, an increased expression of endothelial nitric oxide synthases, VEGF, and nitrotyrosine was found in the gastric wall of rats with prehepatic PH induced by PPVL, indicating the presence of stimuli for further development of collateral circulation.[44]

Aperio[45] or the CAIMAN algorithm[46] may be used to calculate the number of vessels on

images of immunohistochemically stained samples. The programme called AngioPath can quantify microvessels and determine the size and shape of all vessels as well as each vessel individually. As such, it is an important tool for characterising angiogenesis.[47]

Scanning Electron Microscopy

Scanning electron microscopy of vascular casts is helpful for imaging of the splanchnic vascular network's 3D structure with its subsequent quantitative analysis. The technique was used in animals with PPVL and biliary cirrhosis and revealed the presence of newly formed tortuous vessels serving as shunts between the branches of the inferior vena cava and the portal vein. In addition, holes were found in the walls of some capillaries, serving as a sign of intussusceptive angiogenesis, which is one of the two known types of microvessel growth.[8]

ASSESSMENT OF PORTOSYSTEMIC SHUNTING

Portosystemic Shunting Assay Using Microspheres

In 1981, Chojkier and Groszmann[48] proposed to use ^{51}Cr-labelled microspheres to assess the degree of portosystemic shunting. The modification of Chojkier and Groszmann's[48] technique through the use of colour polystyrene fluorescent microspheres has become widespread at the present time, because this technique excludes any contact with the dangerous radioactive material and maintains accuracy.[49]

Approximately 30,000 yellow microspheres (15 µm in diameter) are slowly injected into the spleen. An injection of microspheres of a different colour into the ileocolic vein should be completed for a more detailed haemodynamic assessment of total shunting from the splanchnic area.[50] The liver and lungs of the animal models were removed and placed in centrifuge tubes. Approximately 3,000 blue microspheres were added as an internal control. The tissue was digested in unilocular potassium hydroxide at 60°C for 12 hours and then sonicated. After centrifugation, the supernatant was removed and the pellets were washed once in 10% Triton X-100 solution and twice in acidified ethanol. The precipitate containing microspheres was dried for 12 hours, diluted in acidified Cellosolve™ acetate (The Dow Chemical Company, Midland, Michigan, USA), and the number of microspheres was counted using a spectrophotometer. A haemocytometer and an epifluorescent microscope are also adequate for this procedure.[51] The degree of portosystemic shunting is calculated as the ratio of the number of pulmonary microspheres to their sum in the lungs and liver.[52]

The microsphere technique confirmed that portosystemic collaterals had started forming in rats with PH 2 days after PPVL and became fully developed on Day 7 post PPVL.[28] At the same time, portosystemic collaterals developed later in rats with biliary cirrhosis. Therefore, it is expedient to evaluate them 1 month after bile duct ligation.[53]

Three-Dimensional Micro-Single-Photon Emission Computed Tomography

Since a significant disadvantage of the microsphere method is the need to use laboratory animals, micro-single-photon emission computed tomography (3D micro-SPECT) with technetium (^{99m}Tc) macro aggregated albumin was developed as an alternative. It provides a possibility of conducting serial measurements of portosystemic shunting at different time points after the creation of a model. ^{99m}Tc macro aggregated albumin particles are injected into the splenic pulp; accumulation of the particles in the liver and lungs is determined by using colour scales and computer processing.

3D micro-SPECT was used in mice with PPVL and biliary cirrhosis. The results correlated with the results obtained by using ^{51}Cr-labelled microspheres, and there were no lethality or changes in animals' behaviour after its reusing on the 8th, 12th, and 15th day after the model creation.[54]

FUTURE INSIGHTS INTO ANTIANGIOGENIC THERAPY IN PORTAL HYPERTENSION

Advances in understanding the pathogenesis of PH in cirrhosis stimulated the development of new methods for its pharmacotherapy.

β-adrenoblockers are not recommended for use during the subclinical stage of the disease, while aetiotropic and pathogenetic treatment is reasonable.[55] It may affect, for example, fibro and angiogenesis in the liver (tyrosine kinase inhibitors[56] and statins[57]), as well as angiogenesis that underlies portosystemic shunts formation (tyrosine kinase inhibitors,[58] somatostatin and its analogs,[59] and N-acetylcysteine,[53] among others). This approach, as a part of the complex correction of pathophysiological mechanism contributing to the development of PH, may be the key to success in preventing related complications.

CONCLUSION

The application of modern techniques for studying angiogenesis in experimental research made it possible to establish the important role of new vessel formation in PH pathogenesis. Despite the initially compensatory function, angiogenesis leads to the progression of PH inherent haemodynamic disturbances. The development of antiangiogenic therapy, which would be selectively directed to unusually growing newly formed vessels, may be a promising direction in the treatment of PH and associated complications.

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Management of Non-Small Cell Lung Cancer: The Era of Immunotherapy

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Abstract

Lung cancer is the most frequently diagnosed cancer type and the leading cause of cancer-related deaths worldwide. According to the last GLOBOCAN estimate of cancer incidence and mortality produced by the International Agency for Research on Cancer (IARC), lung cancer accounted for approximately 13% of cancer diagnoses in 2012, and an estimated 1.8 million new lung cancer cases were diagnosed. First-line treatment for Stage IV non-small cell lung cancer (NSCLC) has changed considerably, primarily as a result of a better patient selection on the basis of histology, molecular markers, and innovative treatment approaches.

Recent data have highlighted the advent of immunotherapy as the major shift in treatment of advanced NSCLC. Three checkpoint inhibitors of the programmed death-1–programmed death-ligand 1 interaction, nivolumab, pembrolizumab, and atezolizumab, have already received U.S. Food and Drug Administration (FDA) approval for treatment of advanced NSCLC patients; however, despite impressive treatment responses in many patients who received immunotherapy, a cohort of patients failed to obtain significant results. This review summarises the emerging role of immunotherapy in NSCLC, emphasising the current unanswered questions about predictive biomarkers for treatment response, current treatments, and possible treatment combinations.

INTRODUCTION

The last GLOBOCAN project in 2012 estimated that lung cancer was a leading cause of cancer-related deaths in males worldwide and among females in more developed countries, with an estimated 1.8 million new lung cancer cases diagnosed that year, accounting for approximately 13% of all cancer diagnoses.[1] The emergence of immune checkpoint inhibitors has contributed to an improved prognosis for a large proportion of non-small cell lung cancer (NSCLC) patients. In recent months, the programmed death-1 (PD-1)–programmed death-ligand 1 (PD-L1) checkpoint inhibitors, nivolumab, pembrolizumab, and atezolizumab, have received U.S. Food and Drug Administration (FDA) approval for the treatment of advanced NSCLC. The Phase III KEYNOTE-024 clinical trial evidenced superior efficacy and overall survival (OS) of pembrolizumab compared with platinum-doublet chemotherapy in untreated NSCLC

patients with high PD-L1 expression (≥50% of tumour cells), promoting immunotherapy approaches as a new standard of care for advanced NSCLC.[2-7] This review summarises the emerging role of immunotherapy in NSCLC, highlighting uncertainties regarding predictive biomarkers for treatment response, current treatments, and possible treatment combinations.

IMMUNOTHERAPY IN LUNG CANCER

For a long time, lung cancer was considered a non-immunogenic neoplasm. There have been several randomised clinical trials using the Bacillus Calmette-Guérin (BCG) vaccine, interleukin-2 alone or in combination with other cytokines, or interferon-α alone or in combination with chemotherapy; all showed unfavourable results.[8-10] Recent research has confirmed that lung cancer involves genetic and/or epigenetic alterations that can lead to the generation of neoantigens, known as fragments of mutated proteins displayed in the major histocompatibility complexes of tumour cells critical for the anti-tumour immune response.[11]

PROGRAMMED DEATH-LIGAND 1: PROMISES AND LIMITATIONS

Increasing evidence suggests that the predominant mechanism by which lung cancer cells evade the host's immunological response is through the expression of PD-L1, also called B7-H1 or CD274.[12] PD-1 is an immune-regulatory receptor expressed on the surface of activated T cells, B cells, and natural killer cells. The PD-1–PD-L1 interaction inhibits T cell responses, induces apoptosis of tumour-specific T cells, promotes differentiation of CD4+ T cells into regulatory T cells, and promotes tumour cell resistance.[13-15]

Clinical trials showed that anti-PD-1 and anti-PD-L1 antibodies produced durable responses in approximately 20% of unselected patients with advanced NSCLC.[4,16] It was thought that these receptors would be good candidate biomarkers for selecting patients who were more responsive to immunotherapy; however, not all PD-L1-positive patients are likely to respond to treatment and, more importantly, some patients who test negative for the antibodies may still respond, making it an imperfect biomarker.[17]

In addition to previous considerations, questions have been raised about the technical aspects of PD-L1 testing; these factors included the specificity of several clones of anti-human PD-L1 antibodies for immunohistochemistry (IHC) and the artefacts that may be derived from different techniques for tissue fixation and antigen retrieval.[18] Most of these technical concerns were clarified by IHC assay standardisation but, even with standardised reagents, tissue processing, and test performance, it is difficult to obtain a dichotomous result from a PD-L1 assay since there is no consensus on the level of PD-L1 required to separate positive from negative results.[13] In fact, based on KEYNOTE-001 findings, it is still unclear if PD-L1 can be expressed as a continuous measure rather than a binary positive or negative result. Along with these uncertainties, studies have shown varied results, finding that PD-L1 positivity indicated a favourable, unfavourable, or non-existent relationship to prognosis, as well as variable correlations with histology and mutation status in NSCLC and other tumour types.[13,19] PD-L1-positive values in different reported series ranged from 1–50%, making it difficult to compare results across different studies.[16]

To help solve this issue, an industrial–academic collaborative partnership of drug manufacturers and representatives from Dako and Ventana, the FDA, the American Association of Cancer Research (AACR), the American Society of Clinical Oncology (ASCO), and the International Association for the Study of Lung Cancer (IASLC) was formed. The Blueprint PD-L1 IHC Assay Comparison Project was launched to provide information on analytical and clinical comparability of four PD-L1 IHC assays used in clinical trials. In particular, 22C3 (Dako, Carpinteria, California, USA), 28-8 (Dako), SP142 (Ventana Medical Systems, Tucson, Arizona, USA), and SP263 (Ventana Medical Systems) assays were evaluated; 22C3, 28-8, and SP142 were closely aligned on tumour cell staining and were therefore used, whereas SP263 showed consistently fewer stained tumour cells. The authors evidenced that despite similar analytical performance of PD-L1 expression for the three assays, interchanging assays and

cut-off limits would lead to misclassification of PD-L1 status for some patients.[20]

Despite the development and criticisms of these commercial IHC assays, the role of PD-L1 as a predictive biomarker was also confounded by multiple unresolved biological issues, including different expressions in primary versus metastatic biopsies, oncogenic versus induced PD-L1 expression, intratumour heterogeneity, and staining of tumour cells versus immune cells. In particular, PD-L1 can be expressed by both tumour and inflammatory cells within the tumour microenvironment but the relative importance of either is still unclear; there is no consensus about the relevance of geographic patterns of expression (e.g., proximity of PD-L1 to immune-infiltrating lymphocytes or membranous versus cytoplasmic PD-L1) and PD-L1 evidence may also be affected by concurrent or prior treatment, including radiation or chemotherapy.[12,13]

Considering these data, reliable biomarkers to predict response and select patients to receive anti-PD-1 or anti-PD-L1 treatments are still lacking. It has been suggested that knowledge about PD-L1 expression needs to be applied in the context of T cell infiltrates being blocked by PD-1 receptor engagement.[21] Tumeh et al.[18] demonstrated that CD8 tumour-infiltrating lymphocytes (TIL) in the tumour microenvironment were associated with increased responsiveness to PD-1 inhibition. In this context, Teng et al.[22] classified tumours as Type I (PD-L1-positive with TIL driving adaptive immune resistance), Type II (PD-L1-negative with no TIL indicating immune ignorance), Type III (PD-L1-positive with no TIL indicating intrinsic induction), or Type IV (PD-L1-negative with TIL indicating the role of other suppressors in promoting immune tolerance). Ock et al.[23] comprehensively analysed the data on immunogenomic properties of tumours described in The Cancer Genome Atlas (TCGA) and classified the tumours based on PD-L1 status and TIL. The authors evidenced that high PD-L1 and CD8A expression (Type I tumour) was associated with a high mutational burden, PD-L1 amplification, and oncogenic viral infection. They also concluded that even when considering the cut-off of PD-L1 and TIL recruitment (assessed by CD8A) needed for clinical validation and further confirmation, this integrative analysis highlighted the importance of the assessment of both PD-L1 expression and TIL recruitment to predict responders to immune checkpoint inhibitors.[23]

OTHER PREDICTIVE FACTORS

NSCLC is associated with increased genomic instability and consequential mutations have the potential to generate tumour-specific antigens. Rizvi et al.[24] evaluated the whole-exome sequencing of NSCLC patients treated with pembrolizumab. The authors showed a significantly improved efficacy of anti-PD-1 treatment for NSCLC with high non-synonymous mutation burden in terms of objective response rates (ORR) and durable clinical benefit (partial response or stable disease lasting ≥6 months). In one responder, neoantigen-specific CD8+ T cell responses matched tumour regression, suggesting that anti-PD-1 therapy could enhance neoantigen-specific T cell reactivity.[24] This observation was consistent with the hypothesis that efficacy of anti-PD-1 therapy is largely related to recognition of neoantigens and it is expected to be higher in tumours with a high mutational load, particularly if >10 somatic mutations per megabase pair are present (corresponding to 150 nonsynonymous mutations within expressed genes).[12,25]

Smoking-induced lung cancers are also characterised by a higher number of mutations per megabase pair compared to tumours of never smokers. Govindan et al.[26] described a median of 10.5 mutations per megabase pair (range: 4.9–17.6) in smokers and a median of 0.6 (range: 0.6–0.9) in never smokers. In this context, Rizvi et al.[24] evidenced a greater benefit for tumours harbouring the molecular 'smoking signature', termed transversion-high (TH), compared to those with transversion-low (TL) tumours (ORR: TH 56% versus TL 17%; p=0.03; durable clinical benefit: TH 77% versus TL 22%; p=0.004; progression free survival [PFS]: TH median not reached [NR] versus TL 3.5 months; p=0.0001). In addition, KEYNOTE-001 trial investigators evidenced a response rate to pembrolizumab of 22.5% in current or former smokers compared to 10.3% in never smokers, further supporting that higher mutational burden associated with smoking contributes to an improved response to PD-1

inhibition.[7] Finally, the hypothesis of a role for tumour mutation load and neoantigens in predicting the response to anti-PD-1 treatments is supported by recent evidence that tumours with mismatch-repair-deficiency achieve higher ORR and OS compared to mismatch-repair-proficient tumours.[27]

The presence of *EGFR* mutations and *ALK* rearrangements, which are usually associated with a lack of tobacco exposure, were associated with lower ORR to PD-1 inhibitors. Particularly, Gainor et al.[28] evaluated 58 NSCLC patients treated with PD-1/PD-L1 inhibitors: objective responses were observed in 3.6% of EGFR-mutant or ALK-positive patients versus 23.3% of *EGFR* wild-type and *ALK*-negative or unknown patients (p=0.053). In addition, the ORR measured in never or light smokers (≤10 pack-years) was 4.2% versus 20.6% among heavy smokers (>10 pack-years; p=0.123). When studying advanced *EGFR*-mutant (n=68) and *ALK*-positive (n=27) patients, PD-L1 expression was observed in 24.0%, 16.0%, and 11.0% when cut-off values of ≥1.0%, ≥5.0%, and ≥50.0% tumour cell staining, respectively, were used; PD-L1 expression was also observed in 63.0%, 47.0%, and 26.0% of pre-tyrosine kinase inhibitor (TKI) biopsies using the same tumour call staining cut-offs, respectively.[28] PD-L1 expression levels changed after resistance in 16 (28.0%) *EGFR*-mutant patients with paired, pre, and post-TKI-resistant biopsies (n=57), and concurrent PD-L1 expression (≥5.0%) and high levels of CD8+ TIL (Grade ≥2) were observed in 1 pretreatment (2.1%) and 5 resistant (11.6%) *EGFR*-mutant specimens; this finding was not noted in any *ALK*-positive, pre, or post-TKI specimens.[28] Low rates of concurrent PD-L1 expression and CD8+ TIL within the tumour microenvironment can explain the low ORR to PD-1/PD-L1 inhibitors in NSCLC harbouring *EGFR* mutations or *ALK* rearrangements. Trials are ongoing to test if ORR to immunotherapy can be improved in these tumours if given in concurrence with TKI treatment.[28]

Table 1: Selected programmed death-1 or programmed death-ligand 1 inhibitors in advanced development of non-small cell lung cancer.

Compound	Company	Target	Class	FDA approval
Nivolumab	Bristol-Myers Squibb (New York City, New York, USA)	PD-1	IgG4 fully human Ab	Approved for metastatic NSCLC patients with disease progression during or after platinum-based chemotherapy.
Pembrolizumab	Merck (Kenilworth, New Jersey, USA)	PD-1	IgG4 humanised Ab	Approved for: • Metastatic NSCLC patients whose tumours present high PD-L1 expression (TPS ≥50%) as determined by an FDA-approved test, with no *EGFR* or *ALK* genomic tumour aberrations and no prior systemic chemotherapy treatment for metastatic NSCLC. • Metastatic NSCLC patients whose tumours express PD-L1 (TPS ≥1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with *EGFR* or *ALK* genomic tumour aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab. • Metastatic untreated NSCLC patients in combination with pemetrexed and carboplatin, irrespective of PD-L1 expression.
Atezolizumab	Genentech/Roche (San Francisco, California, USA)	PD-L1	IgG1 engineered Ab	Approved for metastatic NSCLC patients with disease progression during or after platinum-based chemotherapy.

Ab: antibody; FDA: U.S. Food and Drug Administration; Ig: immunoglobulin; NSCLC: non-small cell lung cancer; PD-1: programmed death-1; PD-L1: programmed death-ligand 1; TPS: tumour proportion score.

Adapted from Herzberg et al.[21]

CURRENT IMMUNOTHERAPY APPROACHES AND FUTURE DIRECTIONS

Immunotherapy was approved for NSCLC treatment after impressive and durable responses, a low toxicity profile, and impact on OS as emerged from large randomised Phase III clinical trials.[3,6,7] Immune checkpoints are proteins on lymphocyte surfaces and other immune cells, most notably on cytotoxic T cells that are able to induce stimulatory or inhibitory signals to trigger or reduce cellular adaptive immune responses when bound to their specific ligands.[13] Many checkpoints have been described, including CTLA-4, PD-1 (and its ligand, PD-L1), B7-H3, B7x, T cell immunoglobulin (Ig) and mucin domain-containing molecule-3, and B and T cell lymphocyte attenuators.[29] To date, the best characterised and most clinically studied are PD-1 and PD-L1 inhibitors, as shown in Table 1,[21] as well as CTLA-4 checkpoints.[13]

PROGRAMMED DEATH-1 CHECKPOINT INHIBITORS

Nivolumab

Nivolumab is a fully human IgG4 immune checkpoint inhibitory antibody that binds to PD-1 and prevents its interaction with PD-L1 and PD-L2 (also called B7-DC or CD273). Following a Phase I pilot study by Brahmer et al.,[30] nivolumab has been evaluated in Phase III CHECK-MATE-017 and CHECK-MATE-057 trials.[2,3,30] CHECK-MATE-017 compared nivolumab to docetaxel in 272 advanced, pre-treated, squamous (SQ) NSCLC patients where PD-L1 positivity was not a requirement. This trial led to the first FDA approval of an immunotherapy for NSCLC, with a median OS of 9.2 months in the nivolumab arm (95% confidence interval [CI]: 7.3–13.3) versus 6.0 months in the docetaxel arm (95% CI: 5.1–7.3) and an ORR of 20% versus 9% (p=0.008). Median duration of response was NR with nivolumab (range: 2.9–20.5 months [an ongoing response at the time of analysis]) when compared with 8.4 months for docetaxel (range: 1.4 [with censored data because the patient received subsequent therapy] to 15.2 [an ongoing response at the time of analysis]).[3]

The CHECK-MATE-057 trial[2] evaluated nivolumab versus docetaxel in 582 advanced, pretreated, non-SQ NSCLC patients. Median OS was, again, longer with nivolumab (12.2 months; 95% CI: 9.7–15.0) compared to docetaxel (9.4 months; 95% CI: 8.0–10.7). The ORR was also higher with nivolumab than docetaxel (19.0% versus 12.0%; p=0.02).[2] PD-L1 was assessed retrospectively in prospectively collected pretreatment tumour biopsy specimens from the 582 patients who underwent randomisation; 455 (78%) had quantifiable PD-L1 expression. Cases of PD-L1-negative non-SQ NSCLC did not show a significant benefit with immunotherapy compared with the effect of chemotherapy that was seen in the SQ population (<1% PD-L1 OS HR: 0.90 [95% CI: 0.66–1.24]; <5% PD-L1 OS HR: 1.01 [95% CI: 0.77–1.34]; and <10% PD-L1 OS HR: 1.00 [95% CI: 0.76–1.31]).[2]

Pembrolizumab

Pembrolizumab is a humanised IgG4 monoclonal antibody targeting PD-1. The efficacy and safety of pembrolizumab was first assessed in NSCLC in the Phase I KEYNOTE-001 study by Garon et al.[4] in 2015. Then, KEYNOTE-010,[31] a randomised Phase III trial analogous to CHECKMATE-017 and 057, compared pembrolizumab at two doses, 2 mg/kg and 10 mg/kg every 3 weeks, to docetaxel in 1,034 patients. Only patients with ≥1% PD-L1-positive staining were enrolled, with 593 patients stratified by PD-L1 positivity using a 50% cut-off. Patients treated with pembrolizumab had a higher median OS than with docetaxel (10 mg/kg: 12.7 versus 8.5 months; HR: 0.61; p<0.0001; and 2 mg/kg: 10.4 months; HR 0.71; p=0.0008). When stratified by PD-L1 positivity, defined as a tumour proportion score ≥50%, survival benefits were more pronounced (HR: 0.50 and 0.54 for 10 mg/kg and 2 mg/kg cohorts, respectively), with a median survival of 17.3 and 14.9 months, respectively. In the subgroup analysis, both SQ and non-SQ histology favoured pembrolizumab, consistent with results from nivolumab clinical trials.[31] Based on the unprecedented survival achieved in the PD-L1-positive population, the FDA approved pembrolizumab for second-line therapy in patients with a PD-L1 tumour proportion score ≥1%.

In KEYNOTE-021,[32] front-line pembrolizumab was combined with carboplatin plus pemetrexed

leading to an objective response in 55% of all patients; specifically, an objective response was observed in 54% of patients with ≥1% PD-L1 expression and in 80% of those with ≥50% PD-L1 expression. Updated results showed a significantly improved PFS with pembrolizumab plus chemotherapy versus chemotherapy alone (HR: 0.54; 95% CI: 0.33–0.88; p=0.0067), with a median (95% CI) PFS of 19 months (8.5–NR) versus 8.9 months (95% CI: 6.2–11.8). Median OS was NR (95% CI: 22.8–NR) for pembrolizumab plus chemotherapy and 20.9 months (14.9–NR) in the chemotherapy arm.[32] These data led to accelerated approval, but continued authorisation for this indication may be contingent upon verification and description of clinical benefit in subsequent confirmatory trials.

Pembrolizumab was finally tested as a first-line therapy for metastatic NSCLC compared to different chemotherapy regimens in the Phase III KEYNOTE-024 trial.[6] Patients (n=154) with ≥50% PD-L1-positive staining and no sensitising *EGFR* mutations or *ALK* rearrangements received pembrolizumab, while 151 patients received the investigator's choice of platinum-based chemotherapy. PFS was the primary endpoint and was significantly longer in the pembrolizumab arm compared to chemotherapy (10.3 versus 6.0 months; HR: 0.50; p<0.001). OS was also significantly better in the pembrolizumab group (HR: 0.60; 95% CI: 0.41–0.89; p=0.005).[6] Based on significant improvements in PFS and OS reported by this study, the FDA approved pembrolizumab as the first-line treatment of metastatic NSCLC patients whose tumours express PD-L1 on ≥50% of tumour cells.

Atezolizumab

Atezolizumab is a fully humanised engineered IgG1 monoclonal antibody against PD-L1. Based on OAK study results,[7] in 2016 the FDA granted approval for its use in patients with advanced NSCLC whose disease progressed when treated with platinum-based chemotherapy. The OAK trial[7] was a Phase III trial enrolling pre-treated SQ and non-SQ NSCLC patients who were randomly assigned to receive either atezolizumab (n=425) or docetaxel (n=425). Median OS was 13.8 months (95% CI: 11.8–15.7) in the atezolizumab arm compared to 9.6 months (95% CI: 8.6–11.2) in the docetaxel arm (HR: 0.73; 95% CI: 0.62–0.87; p=0.0003).[7]

CONCLUSION

Although checkpoint inhibitors have dramatically transformed the management of NSCLC, only a small percentage of patients currently benefit from PD-1 blockade therapy alone.[16] Consequently, in order to increase ORR, novel treatment strategies are now under evaluation, including PD-1/PD-L1 inhibitors combined with other checkpoint inhibitors (e.g., CTLA-4, LAG-3, and T cell Ig and mucin domain-containing molecule-3), co-stimulatory checkpoints (e.g., OX40, GITR, and 4-1BB), immunomodulatory molecules (e.g., indoleamide 2,3-dioxygenase), chemotherapy, vaccines, and radiation.[33]

Hellmann et al.[34] reported findings from the Phase I CHECK-MATE-012 study assessing the combination of nivolumab plus ipilimumab, an anti-CTLA-4 antibody, in advanced chemotherapy-naïve NSCLC; PD-L1 expression was not required for enrolment but it was analysed retrospectively. Results of two dose cohorts were reported: nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg either every 12 weeks or every 6 weeks. Confirmed ORR were described in 18 (47%; 95% CI: 31–64) of 38 patients in the ipilimumab every-12-weeks cohort and 15 (38%; 95% CI: 23–55) of 39 patients in the every-6-weeks cohort. Grade 3–4 treatment-related adverse effects (AE) occurred in 14 (37%) patients in the ipilimumab every-12-weeks cohort and 13 (33%) in the every-6-weeks cohort.

Treatment-related serious AE were reported in 12 (32%) patients in the ipilimumab every-12-weeks cohort and 11 (28%) patients in the every-6-weeks cohort. Treatment-related AE of any grade prompted treatment discontinuation in 4 (11%) patients in the every-12-weeks cohort and 5 (13%) in every-6-weeks cohort. Considering these data, larger studies are needed to establish the efficacy balanced with enhanced toxicity of these combinations.[34]

Finally, CHECKMATE-227,[35] a randomised Phase III study of first-line nivolumab plus chemotherapy versus platinum-doublet chemotherapy, is currently underway. Collective results from these and similar studies will help

give clarity regarding how different combination approaches will compare against checkpoint inhibitor monotherapy and platinum-based chemotherapy in distinct patient populations defined by PD-L1 expression alone. This could be even more important considering only about 30% of NSCLC patients have high (>50%) PD-L1 expression, and optimal first-line treatment still needs to be determined for the remaining 70% of patients with lower or absent PD-L1 expression.[36]

In conclusion, it is crucial to deepen the knowledge of immunological mechanisms and their biological impact. For both treatment optimisation and economic reasons, additional research is needed to identify relevant predictive biomarkers. Immunotherapy is undoubtedly a new option for NSCLC management but results from all ongoing clinical trials will further clarify its best application in terms of combinations or sequences of treatments.

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Practical Diagnosis and Staging of Nonalcoholic Fatty Liver Disease: A Narrative Review

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Abstract

As the rates of obesity increase worldwide, the prevalence of nonalcoholic fatty liver disease (NAFLD) has risen and it is now the most common cause of liver disease in the developed world. A significant proportion of patients with NAFLD develop nonalcoholic steatohepatitis and progressive liver fibrosis, which can lead to cirrhosis and its complications. NAFLD should be suspected in individuals who have central obesity and metabolic risk factors. A diagnosis of NAFLD can be made when patients have evidence of steatosis on imaging or if they have raised liver enzymes with a background of metabolic risk factors, provided other causes of liver disease and excessive alcohol consumption are excluded. Making a specific diagnosis of NAFLD is important so that affected individuals can receive specific treatment and be monitored for its complications. The stage of liver fibrosis is the most important prognostic factor so must be assessed in all patients; a number of simple blood tests and imaging modalities allow accurate fibrosis staging without the need for liver biopsy. The aim of this narrative review is to provide a practical overview relating to the diagnosis and staging of NAFLD using noninvasive tests that are widely available in primary and secondary care.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a disorder characterised by central obesity-associated fat deposition in the liver.[1] With rising obesity rates worldwide, NAFLD has become the most common liver disease, affecting 17–46% of adults in Western countries.[2] There has also been a significant rise in liver-related complications secondary to NAFLD, such as hepatocellular carcinoma and liver failure, which has resulted in increased liver transplant rates and hospital admissions for patients with cirrhosis complications.[3,4]

NAFLD is defined as steatosis affecting >5% of hepatocytes in the absence of a secondary cause, such as the use of steatogenic drugs or harmful levels of alcohol consumption (typically defined as >20 g/day for women and >30 g/day for men).[2] NAFLD is strongly associated with

central obesity and metabolic syndrome; almost all individuals with NAFLD have one or more features of metabolic syndrome.[5] Although most individuals with NAFLD have a BMI that lies in the overweight or obese category, 7% of cases have a normal weight BMI (<25 kg/m^2) but they will usually have evidence of central adiposity.[6]

NAFLD is an umbrella term covering the full spectrum of fatty liver disease, which includes steatosis only (fat without hepatocellular injury; also termed nonalcoholic fatty liver), nonalcoholic steatohepatitis (NASH; fat with hepatocellular injury and inflammation with or without fibrosis), and cirrhosis.[7] Overall, 10–30% of the NAFLD population have NASH,[8,9] and this can progress to cirrhosis. Individuals who have steatosis only, and do not progress to NASH, have a good long-term prognosis with very low rates of advanced liver disease.[10] However, approximately 40% of individuals with steatosis develop NASH in the medium-term (5–10 years) and are at risk of progression to advanced liver disease.[11] Progression is more likely to occur if individuals have diabetes or develop diabetes, or if their body weight progressively increases, with a central pattern of adiposity. Overall, in secondary care cohorts of patients with NAFLD, 25–40% will develop progressive liver fibrosis, ultimately leading to cirrhosis in 10–20% of patients.[11,12]

It has recently been established that the most important predictor of mortality in individuals with NAFLD is the stage of liver fibrosis.[13] Individuals with advanced fibrosis (F3; bridging fibrosis) or cirrhosis (F4) have a significantly increased risk of short-to-medium-term all-cause mortality compared with a control population.[14] Patients with F2 fibrosis (periportal or portal fibrosis) are at an increased risk of mortality in the longer term (>20 years), whereas those with F0–1 fibrosis are not at an increased risk of mortality. Therefore, it is particularly important for clinicians to identify individuals with advanced fibrosis and cirrhosis so they can be screened for complications and managed aggressively to reduce their risk of further progression. It is also important to identify those with F2 fibrosis to try and prevent progression to more advanced liver disease.

Currently, the mainstay of treatment for NAFLD is lifestyle modification aimed at weight loss and increasing activity levels.[15] Weight loss of >5% is associated with improvements in steatosis and inflammation, and >10% weight loss may also reduce liver fibrosis.[16] All forms of exercise (moderate intensity, high intensity, or resistance) can improve steatosis independent of weight loss.[17] There is also evidence from small trials that treatment with high-dose vitamin E (800 IU/day), pioglitazone, or liraglutide improves NASH in some patients, and these treatments are currently available (albeit not licensed for NAFLD) so can be used to treat individuals with NAFLD who are at a high risk of disease progression.[1,2,18] Moreover, several new drugs are currently in Phase III trials,[19-22] having shown positive effects in individuals with NASH in Phase II trials, so it is likely that drugs will be licensed for NAFLD within the next 5 years.

Given the large proportion of individuals with NAFLD in the community, it is important that physicians in primary and secondary care have a robust process in place to diagnose and stage NAFLD. This is particularly critical for identifying individuals with more advanced NAFLD to ensure they receive appropriate treatment and monitoring. The aim of this review is to provide a practical overview relating to the diagnosis and staging of NAFLD using noninvasive tests that are widely available in primary and secondary care. The review will also discuss those at risk of NAFLD and how to diagnose and stage the disease.

WHO IS AT RISK OF NONALCOHOLIC FATTY LIVER DISEASE?

NAFLD is strongly associated with increased body weight and metabolic syndrome; therefore, using BMI, waist circumference, or assessment of metabolic risk factors can identify individuals at risk of NAFLD. European guidelines recommend that individuals with metabolic risk factors should undergo procedures for the diagnosis of NAFLD.[2] Overall, hepatic steatosis is present in >80% of centrally obese individuals and 70–90% of those with Type 2 diabetes mellitus (T2DM).[1] Given the high burden of disease in these patients, it is important for clinicians to proactively look for NAFLD. Subjects with T2DM or full metabolic syndrome are particularly at

risk of more advanced NAFLD. A recent study screened 1,799 diabetic patients for liver fibrosis using transient elastography (TE) and found that 17% of the cohort had advanced fibrosis or cirrhosis and 11% had cirrhosis.[23]

DIAGNOSIS OF NONALCOHOLIC FATTY LIVER DISEASE

Most individuals with NAFLD are asymptomatic and present incidentally with raised liver enzymes or evidence of steatosis on imaging;[24] if symptoms do occur, they include right upper quadrant pain or fatigue. Although liver enzymes are frequently 'normal' in patients with NAFLD, identification of raised liver enzymes often prompts further investigation. The most common liver enzyme abnormality seen in patients with NAFLD is raised gamma-glutamyltransferase (GGT) levels.[25] Raised transaminases are also seen, but 80% of individuals with NAFLD have an alanine aminotransferase (ALT) level within the 'normal' laboratory range (<35–50 U/L depending on the laboratory).[5] The use of a lower normal range for ALT (<30 U/L for males and <19 U/L for females) is more sensitive for NAFLD.[26] However, serum ALT values do not correlate with histological findings in NAFLD and, therefore, ALT cannot be used to make the diagnosis, distinguish between steatosis and steatohepatitis, or quantify the stage of fibrosis.[27,28] Raised liver enzymes, typically elevated ALT and/or GGT, in an individual with obesity or who has metabolic risk factors usually signifies fatty liver, provided other causes of liver disease are excluded (Box 1),[1,24] but clinicians must not rely on liver enzymes to diagnose NAFLD. The finding of a raised serum immunoglobulin A or ferritin with normal transferrin saturation can be supportive of a diagnosis of NAFLD because these abnormalities are present in 46% and 33% of patients with NAFLD, respectively.[29]

To be diagnosed with NAFLD, patients must not regularly consume excessive alcohol (>30 g/day for males or 20 g/day for females). In clinical practice, it is common to encounter patients with fatty liver, metabolic risk factors, or central obesity, who also regularly consume more than this amount of alcohol. These individuals are at a particularly high risk of developing advanced liver disease because steatosis from alcohol and metabolic syndrome appears to act synergistically, leading to a more severe liver injury.[30] Reducing their alcohol intake as well as managing their obesity and metabolic syndrome is the mainstay of management for these individuals.

Once suspected clinically, the presence of hepatic steatosis should be confirmed on imaging.[2] Ultrasound is the first-line investigation for these cases because it is inexpensive and readily available. A fatty liver is associated with increased hepatic echogenicity and appears bright on ultrasound. Ultrasound is accurate for a diagnosis of >20–30% steatosis cases (85% sensitivity and 94% specificity) but is poorly sensitive for mild steatosis.[31] There have been recent developments using semiquantitative ultrasound scores to help improve the diagnostic accuracy of ultrasound;[32] however, this technique remains operator-dependent and is not routinely available in all clinical settings. Therefore, if clinicians have a strong clinical suspicion that a patient has hepatic steatosis and the ultrasound is negative, then further investigations may be required. In this situation, measurement of controlled attenuation parameter (CAP) can be helpful. This is measured contemporaneously with liver stiffness by TE (Fibroscan™, Echosens, Paris, France), which is available in many secondary care centres. CAP provides an estimate of steatosis by measuring changes in the propagation of a shear wave through the liver, which is altered in a steatotic liver. This technique gives a reading of 100–400 dB/m and has been shown to accurately detect >10% and >33% of steatosis (area under receiver operator curve [AUROC]: 0.91 and 0.95, respectively).[33] One study showed that a CAP reading of ≥283 dB/m was 77% accurate for a diagnosis of >10% steatosis.[34]

Magnetic resonance imaging (MRI) is the most accurate noninvasive modality for diagnosing steatosis but is expensive and not easily accessible. Liver fat can be measured using a number of techniques, including the Dixon technique for in-phase and out-of-phase imaging, magnetic resonance spectroscopy, and MRI-proton density fat fraction (MRI-PDFF).[35,36] All of these techniques are quantitative but require specific expertise to interpret the

results. MRI-PDFF, the newest technique, has the advantage of assessing steatosis in the whole liver and it has been shown to correlate well with histological assessment of steatosis.[36] Moreover, changes in MRI-PDFF following treatment for NAFLD correlate with histological changes in liver fat, suggesting that this technique may be useful to monitor treatment response.[37]

In most cases, NAFLD can be confidently diagnosed without the need for a liver biopsy in individuals with steatosis on imaging or in those with raised liver enzymes and metabolic risk factors, after the exclusion of other causes (Box 1). In cases where there is diagnostic uncertainty, a liver biopsy is usually helpful to confirm the diagnosis.

STAGING OF NONALCOHOLIC FATTY LIVER DISEASE

Once a diagnosis of NAFLD has been made, it is vital to stage the disease to assess prognosis and determine if specific treatment (in addition to lifestyle changes) for NAFLD is required. As previously discussed, the stage of fibrosis is the most important prognostic factor in NAFLD so should be assessed in all patients. There have been numerous studies assessing the diagnostic accuracy of a multitude of biomarkers of fibrosis in NAFLD;[38] however, many of these biomarkers are not widely available or remain under investigation, so discussion here will focus on the routinely available tests.

Simple Noninvasive Fibrosis Scoring Systems

Several simple noninvasive scoring systems for fibrosis have been assessed and validated in NAFLD. These are derived from routinely available laboratory tests (ALT, aspartate transaminase [AST], platelets, or albumin) and clinical measurements (such as age, BMI, and presence of T2DM), and have the advantage of being inexpensive and widely available. Some of the more widely used scoring markers are discussed in Table 1.

Aspartate Transaminase/Alanine Aminotransferase Ratio

The simplest score is the AST/ALT ratio (AAR).[39] As fibrosis progresses to advanced fibrosis or cirrhosis, the serum ALT level typically falls, while the AST level remains stable or increases, resulting in an increased AAR.[27,44] One study in patients with NAFLD found that an AAR <0.8 could exclude advanced fibrosis with reasonable accuracy (AUROC: 0.83; negative predictive value [NPV]: 93%).[27] However, more complex models that incorporate the AAR, such as the Fibrosis-4 (FIB-4) score or NAFLD fibrosis score (NFS), are generally more accurate.

Box 1: Main differential diagnoses that should be excluded when investigating nonalcoholic fatty liver disease.

Differential diagnosis	Key history or investigation to exclude differential diagnosis
ARLD	History of excessive alcohol consumption (>20 g/day for women and >30 g/day for men); liver biopsy is frequently not helpful in distinguishing ARLD from NAFLD.
Hepatotoxic drugs	Medication history of known hepatotoxic drugs, such as methotrexate, tamoxifen, amiodarone, and others.
Viral hepatitis	Hepatitis C antibody, hepatitis B surface antigen.
Autoimmune hepatitis	Positive liver autoantibody screen, raised immunoglobulin G, and clinical history of other autoimmune conditions.
Haemochromatosis	Raised ferritin with transferrin saturation >45%.
Coeliac disease	Positive TTG antibody.
Wilson's disease	Low caeruloplasmin and low alkaline phosphatase.
Alpha-1 antitrypsin deficiency	Low alpha-1 antitrypsin and alpha-1 antitrypsin phenotype.
Cholestatic disorders	Typically has a cholestatic pattern of liver enzymes (raised alkaline phosphatase and GGT).

ARLD: alcohol-related liver disease; GGT: gamma-glutamyltransferase; NAFLD: nonalcoholic fatty liver disease; TTG: tissue transglutaminase.

Table 1: An overview of the simple noninvasive fibrosis markers and their limitations.[27,39-43]

Test	Calculation method	Lower cut-off*	Upper cut-off*	Limitations
AST/ ALT ratio	AST/ALT	0.800	1.000	• Inaccurate for those <35 years old. • Reduced specificity with increased age. • Less accurate than FIB-4 and NFS.
FIB-4 score	Age x AST (IU/L)/platelet count (x10^9/L) x √ALT (IU/L)	1.300 (<65 years) 2.000 (≥65 years)	2.670	• Inaccurate for those <35 years old. • Reduced specificity with increased age. • Unreliable in individuals of South Asian descent. • Reduced performance in obesity surgery cohorts. • Reduced performance in those with myositis (↑AST) or platelet disorders (↓or↑). • 28% of patients fall into the indeterminate category.
NFS	-1.675 + 0.037 x age (years) + 0.094 x BMI (kg/m^2) + 1.13 x impaired fasting glycaemia or diabetes (yes=1, no=0) + 0.99 x AST/ALT ratio - 0.013 x platelet (x10^9/L) -0.66 x albumin (g/dL)	-1.455 (<65 years) 0.120 (≥65 years)	0.676	• Inaccurate for those <35 years old. • Reduced specificity with increased age. • Unreliable in individuals of South Asian descent. • Reduced performance in obesity surgery cohorts. • Reduced performance in those with myositis (↑AST), platelet disorders (↓or↑), or protein losing states. • High false-positive rate in individuals with normal liver enzymes or if used to screen for advanced fibrosis in diabetes clinics. • 25% of patients fall into the indeterminate category.

*A score less than the lower cut-off excludes advanced fibrosis and a score above the upper cut-off diagnoses advanced fibrosis. Scores between the lower and upper cut-offs are indeterminate.

ALT: alanine aminotransferase; AST: aspartate transaminase; FIB-4: Fibrosis-4 score; NFS: nonalcoholic fatty liver disease fibrosis score.

Nonalcoholic Fatty Liver Disease Fibrosis Score

The NFS was developed to diagnose or exclude advanced fibrosis (F3–4) cases in a multicentre cohort of 733 patients with NAFLD.[40] The model includes age, BMI, AAR, presence of T2DM or impaired fasting glycaemia, platelet count, and albumin, and can easily be calculated using an online calculator (Table 1). This model uses two diagnostic cut-offs: a score of <-1.455 can exclude advanced fibrosis with reasonable accuracy (NPV: 88–93%), while the higher cut-off of >0.676 can detect advanced fibrosis with some accuracy (positive predictive value: 82–90%).[40] Individuals with an indeterminate score usually require a second-line fibrosis assessment. The NFS has been validated in multiple cohorts worldwide[41,45-47] and has also been shown to predict mortality (all-cause, liver, and cardiovascular) in patients with NAFLD.[48]

Fibrosis-4 Score

This score was originally developed in a cohort of individuals with hepatitis C virus/HIV coinfection and is also effective in patients with NAFLD.[41,42] The model includes age, ALT, AST, and platelet count (Table 1). A score of <1.3 reliably excludes advanced fibrosis (NPV: 90–95%), while a score of >2.67 is strongly suggestive of advanced fibrosis (positive predictive value: 80%).[27,41] Only 28% of patients fall into the unclassified category.[27,41] The FIB-4 score has also been externally validated in many cohorts,[45-47] including patients with NAFLD and normal ALT levels.[49] Overall, the FIB-4

score performed slightly better than the NFS in most studies and requires fewer variables to calculate, therefore making it the most simple noninvasive score of choice in our unit.

BARD Score

This score includes the weighted sum of BMI (>28 kg/m^2=1 point), AAR (>0.8=2 points), and the presence of T2DM (present=1 point).[44] A score of <2 has an excellent NPV of 95–97% in excluding advanced fibrosis (F3-4); however, the false-positive rate for a score of ≥2 is high, which limits its use in clinical practice.[27]

Aspartate Transaminase to Platelet Ratio Index

The aspartate transaminase to platelet ratio index has been extensively investigated in patients with hepatitis C virus and a score of >1 is suggestive of cirrhosis. However, the performance of the index was suboptimal (AUROC: 0.67 for diagnosis of F3–4) when compared with other simple noninvasive markers in patients with NAFLD.[27]

Limitations of Simple Noninvasive Fibrosis Scores in Nonalcoholic Fatty Liver Disease

The NFS and FIB-4 scores are the most widely used simple noninvasive scores and are recommended in European guidelines.[2] However, these scores do have some limitations (Table 1), which are important to consider when using them in clinical practice. Firstly, these scores have been derived to exclude or diagnose advanced fibrosis or cirrhosis (F3–4) and have limited ability to detect earlier stages of fibrosis. Both the FIB-4 score and NFS have good NPV and can therefore reliably exclude advanced fibrosis; as a result, their main role is to identify lower risk patients who may not need referral to secondary care.[27] However, both scores have a high false-positive rate for advanced fibrosis, so further investigations to confirm advanced fibrosis are often required in those with indeterminate or high scores.

It has been recently appreciated that age is an important factor affecting the accuracy of the FIB-4 score and NFS. One study showed that the NFS and FIB-4 score performed poorly in patients with NAFLD aged <35 years (AUROC: 0.52 and 0.60 for F3 and F4, respectively), probably due to the low prevalence of F3 or F4 scores in that age group.[50] Therefore, alternative strategies are needed to stage fibrosis in this age group. In the same study, it was shown that the specificity for advanced fibrosis with the NFS and the FIB-4 scores decreased with age, becoming unacceptably low in those aged >65 years (for F3 and F4, 20% and 35%, respectively, compared to 80% and 91% in patients 36–45 years old, respectively). As a result, new cut-offs have been suggested to reduce the false-positive rate for F3–4 fibrosis in those aged >65 years (<2.0 for FIB-4 and <0.12 for NFS). These cut-offs have been incorporated into UK guidelines.[51]

It has also been shown that simple noninvasive fibrosis tests perform differently in some ethnic groups. One study found that the NFS and FIB-4 score have reduced sensitivity for significant or advanced fibrosis in individuals of South Asian ethnicity, possibly due to the high rates of obesity and T2DM in this population.[52] Alternative diagnostic cut-offs may address this problem. Simple noninvasive fibrosis scores may also have reduced performance in cohorts of patients without known liver disease undergoing obesity surgery.[53] Moreover, it remains unknown how well these scores perform in asymptomatic individuals in the community with incidentally identified fatty liver and normal levels of liver enzymes.

Overall, despite their limitations, the FIB-4 score and NFS offer a good first-line test to stage liver fibrosis and if individuals have a low score (using appropriate age corrected cut-offs), advanced fibrosis can reliably be excluded. For those with an indeterminate or high score, it is usually appropriate to conduct a second-line test to confirm advanced fibrosis.

Biomarkers and Commercial Fibrosis Panels

A number of biomarker panels for fibrosis have been studied in patients with NAFLD, with a few used routinely in some centres. Given their expense compared with simple noninvasive tests, biomarker panels are often used as a second-line.

The Enhanced Liver Fibrosis (ELF™) Test

This commercial panel includes tissue inhibitor of metalloproteinase-1, hyaluronic acid, and amino-terminal propeptide of Type III procollagen and is marginally more effective than the NFS for measuring advanced fibrosis (F3–4) in patients with NAFLD (AUROC: 0.93 versus 0.89).[54] An advantage of the ELF™ test (Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA) over the NFS and FIB-4 score is that it has a single cut-off for advanced fibrosis, which eliminates indeterminate results, but it is expensive when compared with simple fibrosis markers. The National Institute of Health and Care Excellence (NICE) recommends the ELF test first-line to stage fibrosis in patients with NAFLD. However, it is more commonly used as a second-line after simple noninvasive scores.[55]

FibroTest

The FibroTest is a commercial panel, commonly used in France, including total bilirubin, GGT, α2-macroglobulin, apolipoprotein A1 and haptoglobin, age, and sex. It has been validated in several liver diseases, including NAFLD where it has acceptable accuracy for advanced fibrosis (F3–4; AUROC: 0.81–0.92).[56]

Imaging Assessment of Fibrosis

Staging of fibrosis with imaging is frequently conducted following an initial triage with simple noninvasive scores or biomarkers. A thorough review of advances in imaging assessment of NAFLD has been recently conducted.[57] Here, we will discuss imaging techniques that are widely available.

Transient Elastography

TE (Fibroscan) is the most commonly used imaging modality for liver fibrosis assessment and has been extensively validated.[58] TE uses ultrasound to assess liver elasticity by measuring the velocity of a shear wave through a region of interest in the liver. The liver stiffness measurement correlates well with fibrosis stage in a range of liver diseases, including NAFLD. Two probes are available for use in adults: the M probe and the XL probe. Both probes have been validated in patients with NAFLD but give slightly different readings so it is important to use the appropriate diagnostic cut-offs for each probe.[59] A limitation of TE is the failure rate, particularly in obese individuals, although this is reduced with the XL probe.[59,60]

Studies have shown that TE has an excellent ability to exclude advanced fibrosis, with few false-negatives.[59] It also has better positive predictive ability for significant and advanced fibrosis than simple noninvasive tests and, therefore, offers a good second-line test to confirm advanced fibrosis.[61] Although there is still debate over the optimum cut-off for TE, as a general rule <8.0 kPa (or <7.2 kPa for the XL probe) reliably excludes advanced fibrosis (F3-4) and >9.6 kPa suggests F3-4.[57]

It has recently been shown that the degree of hepatic steatosis has an impact on the accuracy of TE; one recent study showed that the false-positive rate for ≥F2 or F3–4 was significantly higher in subjects with severe steatosis compared with those with mild or moderate steatosis.[62] One way to mitigate against this could be to adjust liver stiffness measurement cut-offs according to the patient's CAP value,[63] but further studies are needed to clearly define the diagnostic cut-offs.

Acoustic Radiation Force Impulse Imaging

Standard ultrasound has a very limited ability to stage liver fibrosis other than diagnosing cirrhosis when features of portal hypertension are present. However, many ultrasound machines can now measure liver elasticity using acoustic radiation force impulse imaging (ARFI), a form of shear wave elastography. There are a few variations of ARFI available, the best studied in NAFLD being Virtual Touch™ Quantification (Siemens Medical Solutions Inc., Mountain View, California, USA). A meta-analysis showed that ARFI was reasonably accurate in diagnosing significant fibrosis (≥F2; AUROC: 0.898).[64] However, optimum diagnostic cut-offs have not been determined. Another method of ultrasound elastography, supersonic shear imaging, has also shown promise, performing slightly better than TE and ARFI in a recent study of 291 patients with biopsy-proven NAFLD,[65] but further validation is required.

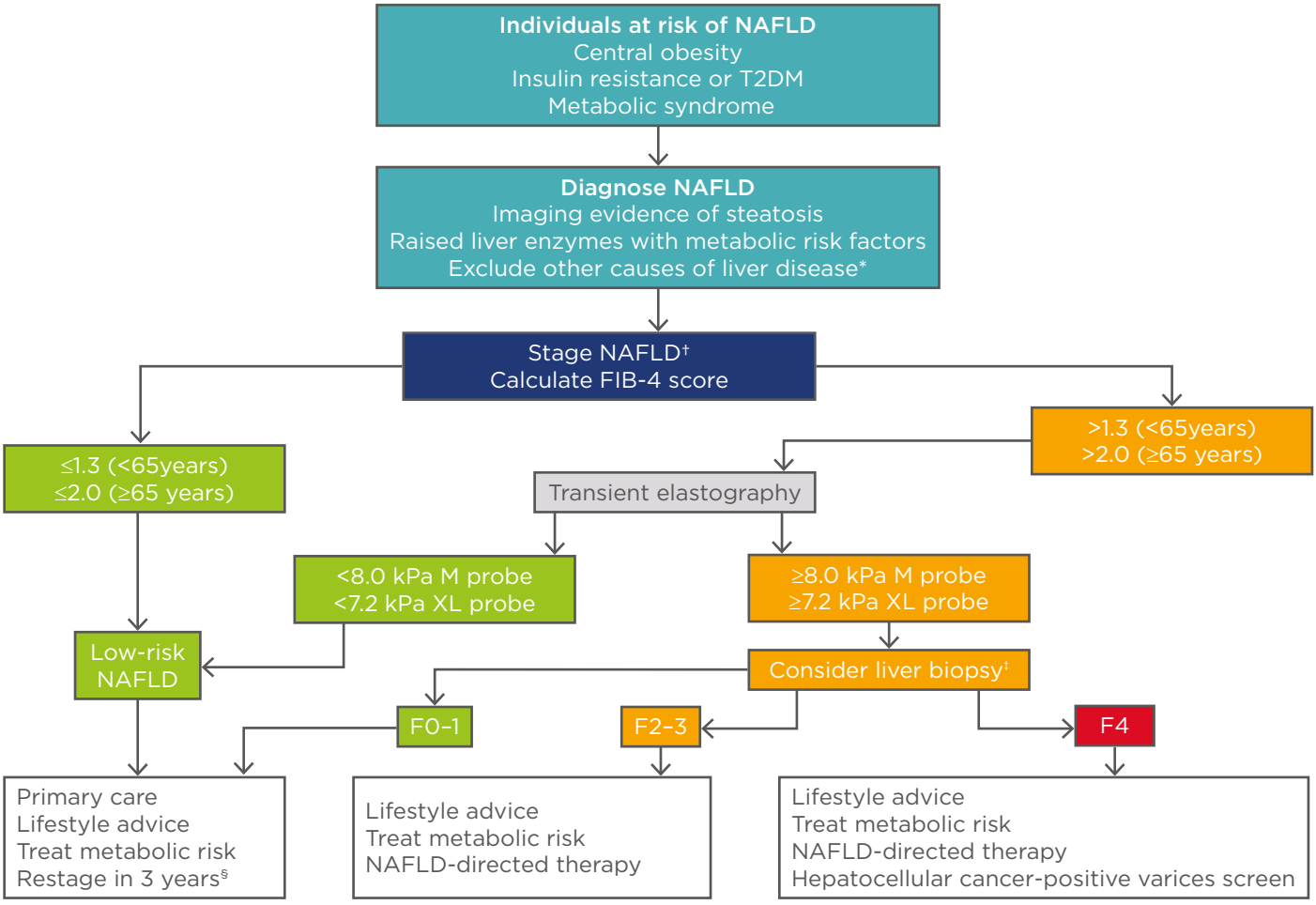

Figure 1: A potential algorithm for the diagnosis and staging of nonalcoholic fatty liver disease.

*Other causes include harmful alcohol consumption, hepatotoxic medications, viral hepatitis, autoimmune liver disease, haemochromatosis, coeliac disease, Wilson's disease. †The FIB-4 score is unreliable in young patients (<35 years) so consider transient elastography first-line. ‡A liver biopsy is required to diagnose NASH if patients are to receive specific NASH treatment. §Restage with FIB-4 if previously low or transient elastography if FIB-4 was previously high.

FIB-4: Fibrosis-4 score; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; T2DM: Type 2 diabetes mellitus.

Magnetic Resonance Elastography

Magnetic resonance elastography is considered the most accurate noninvasive test for liver fibrosis in patients with NAFLD.[66] This technique measures elasticity in the whole liver using a modified phase-contrast pulse sequence that allows visualisation of a transmitted shear wave in the liver tissue. Although not widely available, studies have shown that magnetic resonance elastography is superior to TE in patients with NAFLD, particularly for the diagnosis of early-stage fibrosis.[67,68] This technology is currently being assessed as a potential outcome measure for therapeutic clinical trials.

Biomarkers of Steatohepatitis

Although fibrosis stage is the key factor associated with long-term prognosis, identifying individuals with active steatohepatitis is important because hepatocellular injury leads to the development of fibrosis and the treatment of steatohepatitis reduces the risk of disease progression. Currently, there are no validated biomarkers that can reliably diagnose NASH in an individual patient. One previous study suggested that elevated serum cytokeratin-18 levels, a marker of hepatic apoptosis, may indicate the presence of NASH;[69] however, subsequent studies found that cytokeratin-18

levels may not help with diagnosis in individual patients.[70] A number of other biomarkers of steatohepatitis are under investigation at present.[38] Currently, the only way of reliably diagnosing NASH is by liver biopsy.[2]

CONCLUSION

With rising rates of obesity, NAFLD has become very common, affecting 20–30% of the general population.[7,15,21] Although the majority of those with NAFLD have mild disease, a significant proportion have advanced liver disease and are at risk of liver-related complications. Clinicians should have a high index of suspicion for NAFLD and follow a diagnostic and staging pathway to identify those who need more specific treatment or surveillance for the associated complications. An example of such a pathway is shown in Figure 1; however, this pathway can be modified depending on the availability of tests locally.

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New Insights into the Role of Phosphoinositide 3-Kinase Activity in the Physiology of Immature Oocytes: Lessons from Recent Mouse Model Studies

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Abstract

The immature oocytes within primordial follicles are arrested at Prophase I of meiosis and remain dormant until awakened by an increase in intracellular levels of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Oocyte PIP3 level is determined by the balance between the activity of phosphoinositide 3-kinase (PI3K) and phosphatase and tensin homologue (PTEN). When this balance favours PI3K, PIP3 levels elevate and trigger the cascade of PI3K/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, leading to activation of primordial follicles. This short review aims to provide new insights into the physiological functions of PI3K and PTEN in immature oocytes by summarising recent findings from murine model studies, including oocyte-specific transgenic mice with constitutively-active mutant PI3K.

OVARIAN RESERVE SIZE IS THE PREDOMINANT DETERMINANT OF FEMALE REPRODUCTIVE LIFESPAN

Female mammals are born with a finite number of oocytes, and the initial pool of primordial follicles (PF) is referred to as the ovarian reserve. The oocytes within the PF are arrested at Prophase I of meiosis and remain quiescent within the ovary until recruited into the growing follicle pool.[1,2] Only a small number of PF are activated in each reproductive cycle, and this selective recruitment of PF is tightly regulated so that the reproductive lifespan is maintained for months in mice and decades in humans. If the activation rate of PF is accelerated, the ovarian reserve in women would be exhausted before reaching the age of expected menopause. The average age of women in the USA at the time of menopause is 51 years. If loss of normal ovarian function occurs before the age of 40 years, the condition is called premature ovarian failure or primary ovarian insufficiency (POI).[3-5]

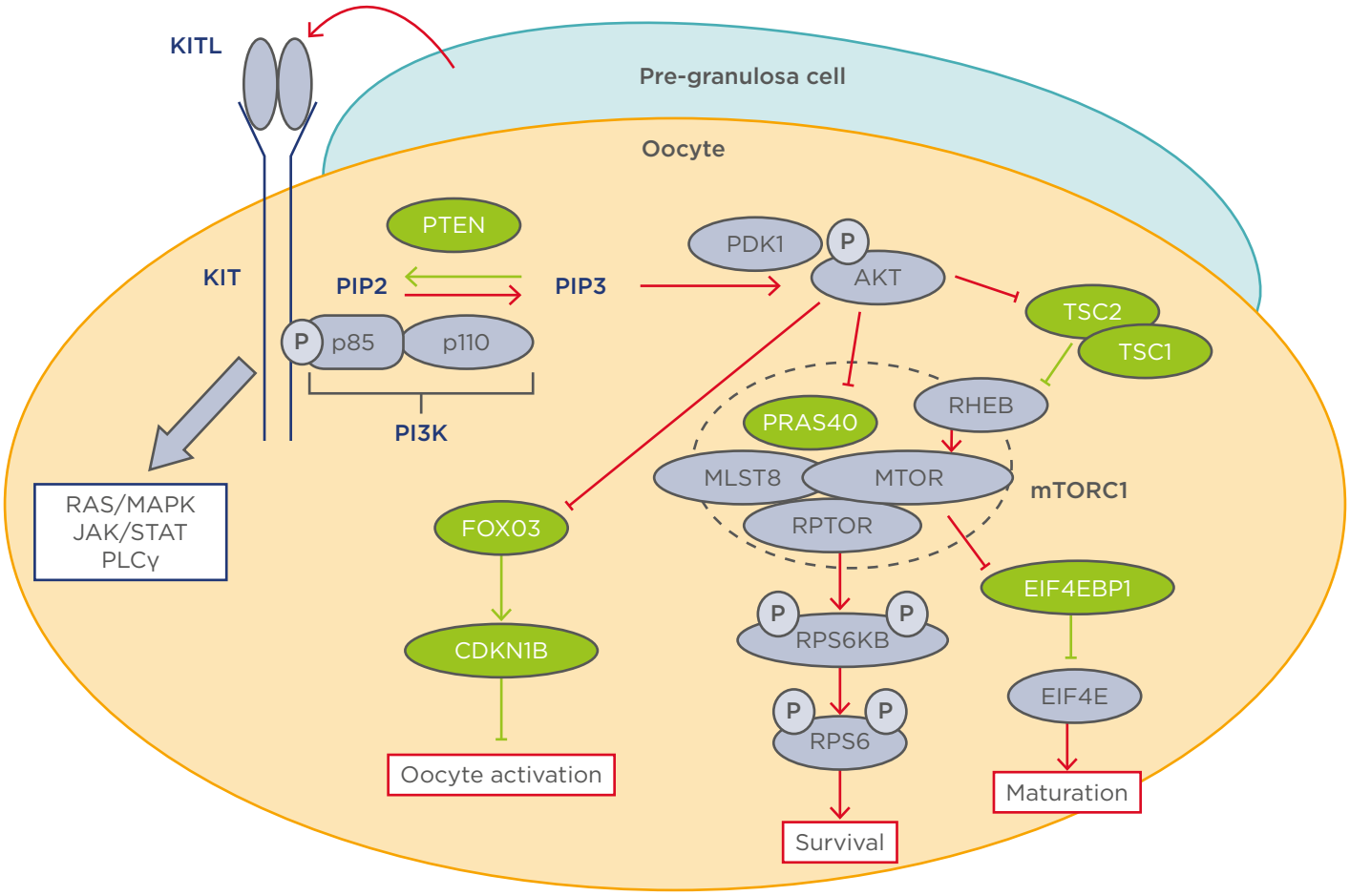

Figure 1: Diagram of the phosphoinositide 3-kinase pathway in oocyte physiology.

Molecules coloured green and purple are negative and positive regulators of the PI3K/AKT/mTORC1 pathway respectively. Red and green lines indicate activation and inhibition of the PI3K/AKT/mTORC1 pathway respectively.[15-18,23-27,32-34,37]

AKT: protein kinase B; CDKN1B: cyclin-dependent kinase inhibitor 1B; EIF4E: eukaryotic translation initiation factor 4E; EIF4EB1: eukaryotic translation initiation factor 4E binding protein 1; FOXO3: forkhead box 03; JAK: janus kinase; KITL1: KIT ligand 1; MAPK: mitogen-activated protein kinase; MLST8: mTOR associated protein, LST8 homologue; mTOR: mechanistic target of rapamycin; mTORC1: mechanistic target of rapamycin; p: phosphate group; PDK1: phosphoinositide-dependent kinase-1; PI3K: phosphoinositide 3-kinase; PLCγ: phospholipase C, gamma; PTEN: phosphatase and tensin homologue; PRAS40: proline-rich AKT1 substrate of 40 kDa; RHEB: Ras homologue enriched in brain; RPS6: ribosomal protein S6; RPS6KB: ribosomal protein S6 kinase B1; RPTOR: regulatory-associated protein of mTOR; STAT: signal transducer and activator of transcription; TSC: tuberous sclerosis.

BALANCE BETWEEN PHOSPHOINOSITIDE 3-KINASE AND PHOSPHATASE AND TENSIN HOMOLOGUE CONTROLS FOLLICLE ACTIVATION

The awakening of oocytes in PF is triggered by an increase in the levels of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) within the oocyte.[6-9] The primary regulators of oocyte PIP3 levels are phosphoinositide 3-kinase (PI3K) and phosphatase and tensin homologue (PTEN), which exist in a counterbalanced relationship; PI3K catalyses phosphatidylinositol 4,5-bisphosphate (PIP2) to PIP3, whereas PTEN converts PIP3 back to PIP2 (Figure 1). When the balance favours PI3K activity, an elevated PIP3 level triggers the cascade of PI3K/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway: the binding of PIP3 to the pleckstrin homology domain anchors AKT to the plasma membrane;

it is then phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1). Phosphorylated AKT then targets multiple that control the activation of PF. Forkhead box O3 (FOXO3) transcription factor is one of the major direct substrates of AKT in immature oocytes. FOXO3 resides in the nucleus of dormant primordial oocytes, repressing the expression of genes that awaken the oocytes. When phosphorylated by phosphorylated AKT, FOXO3 is excluded from the nucleus and the expression or repression of FOXO3 target genes activates the oocyte.[10,11] Phosphorylated FOXO3 remains in the cytoplasm of primary follicles until it is eventually degraded in secondary follicles.[12] AKT also directly phosphorylates tuberous sclerosis (TSC) 2 and relieves the inhibitory effects of the TSC1-TSC2 complex on Ras homologue enriched in brain and mTOR complex 1 (mTORC1).

The critical role of the PI3K/AKT/mTOR pathway in the regulation of PF activation was established by studies in murine genetic models in which the negative regulators of the PI3K/AKT/mTOR pathway are ablated in oocytes.[9,13,14] In the ovaries of oocyte-specific *Pten* conditional knockout (cKO) mice,[15] the ovarian reserve was completely depleted by postnatal day (PD) 28 through spontaneous activation of PF; however, the global premature activation of PF in *Pten* cKO mice was reversed by concomitant loss of PDK1.[16] This study clearly indicates that the activity level of the PI3K pathway determines whether PF is activated or not. Similar to *Pten* cKO mice, oocyte-specific ablation of *Tsc1* and *Tsc2*, the negative regulators of mTORC1, resulted in premature ovarian failure or POI. Both oocyte-specific *Tsc1* cKO and oocyte-specific *Tsc2* cKO mice replicated the phenotypes of oocyte-specific *Pten* cKO mice, and their ovarian reserve was depleted by PD28.[17] Furthermore, *Foxo3* null or oocyte-specific *Foxo3* cKO mice also deplete the ovarian reserve through global premature follicle activation.[11,15] In all null mutant mice for the negative regulators of the PI3K/AKT/mTOR pathway, primary oocytes are activated almost immediately after the formation of PF, indicating that the cue for PF activation is a signal that disrupts the mechanism maintaining oocyte quiescence. Indeed, the oocyte expression of mutant FOXO3, which lacks critical phosphorylation sites for nuclear exclusion, slows down the recruitment of PF, suggesting that inactivation or phosphorylation of FOXO3 is not only sufficient but also essential to activate dormant oocytes.[18] The critical role of PTEN activity in the maintenance of oocyte dormancy is not mouse specific. Dipotassium bisperoxo (5-hydroxypyridine-2-carboxyl) oxovanadate (bpV), a reversible inhibitor of PTEN, activates PF within human ovarian tissue fragments *in vitro*. The bpV treatment was accompanied with AKT phosphorylation and nuclear exclusion of FOXO3.[19] Furthermore, the bpV treatment has led to successful deliveries of healthy babies in POI patients,[14] establishing that mouse genetic models are valid models for human ovarian physiology.

MECHANISMS THAT CONTROL THE SELECTIVE RECRUITMENT OF PRIMORDIAL FOLLICLES

The exact mechanism that controls the selective activation of PF remains elusive. The generally accepted model proposes external signals that target pre-granulosa cells (GC) and/or oocytes trigger the signal transduction, leading to the activation of PI3K within oocytes.[20-22] Using the analogy of an accelerator and brake may help explain the roles of PI3K (accelerator) and PTEN (brake) in PF activation (automobile): when the input of the accelerator exceeds that of the brake, the car moves forward (PF activation). When the brake is broken (PTEN null), the car can still crawl forward even without input from the accelerator, as revealed by spontaneous activation of PF in oocyte-specific *Pten* cKO mice. Furthering this analogy, mouse studies demonstrated the presence of multiple brakes in oocyte activation (e.g., TSC1, TSC2, FOXO3, and cyclin-dependent kinase inhibitor 1B) at different levels of the PI3K/AKT/mTOR pathway,[16,18,22,23] and all brakes are simultaneously required to maintain the dormancy of primordial oocytes; PF are activated if any one of these negative regulators is lost.

In this regard, oocyte PTEN is the master brake maintaining the dormancy of PF because all other brakes in the system are released when PTEN activity is exceeded by the activity of PI3K. In theory, overproduction of PIP3 in oocytes should have the same effect as

the loss of PTEN. Hence, we generated an oocyte-specific transgenic mouse model for a constitutively-active mutant PI3K (PIK3CA*, *oo-Pik3ca** mice) and examined if the phenotypes mimic those of oocyte-specific null mice for PI3K/AKT/mTOR pathway inhibitors.[24] Unexpectedly, overproduction of PIP3 by mutant PI3K was insufficient to activate the PF. Excess PI3K activity was counteracted by an up-regulation of PTEN. Correspondingly, the primordial oocytes retained FOXO3 in the nucleus and remained dormant. In accordance with these findings it was concluded that PTEN is dominant over PI3K activity in primordial oocytes and thus the activation of PF may involve a signal that down-regulates oocytic PTEN.

However, a recent genetic study in mice demonstrated the pivotal roles of the KIT–KIT ligand (KITL) signalling pathway in awakening dormant primordial oocytes, refuting our PTEN-dominant hypothesis. Oocyte-specific expression of the *Kit* Asp818Val allele, which produces a mutant KIT protein equivalent to the oncogenic mutant KIT Asp816Val in humans, spontaneously activated PF.[25,26] Thus, the activation of the PI3K/AKT/mTOR pathway by KIT–KITL signalling is sufficient to awaken the oocytes within PF. The study strongly suggests that the recruitment of PF to the pool of growing follicles is triggered by the interaction between GC and oocytes, mediated by KIT–KITL signals. It raises the question that if activation of the PI3K signalling cascade is sufficient for the activation of PF, why does the expression of constitutively active PI3K not result in POI in mice? There are several nonexclusive explanations. One explanation for the contradictory phenotypes is the different level of PI3K activity and, thus, PIP3 concentrations (Figure 2). In conditional transgenic mice for KIT Asp816Val, the mutant *Kit* was knocked into the endogenous *Kit* locus, such that levels of mutant KIT Asp818Val protein and PI3K activation should be within physiological range. Therefore, the PIP3 levels should be elevated at a rate similar to innate follicle activation. In contrast, our transgenic mice overexpress the mutant PIK3CA* from the *ROSA26* locus; as a result, the acute elevation of PIP3 to an unphysiological rate might have triggered an innate corrective system mediated by upregulation and nuclear accumulation of PTEN (Figure 2). A second explanation is the complexity of KIT signalling pathways. In addition to the PI3K/AKT/mTOR pathway, KIT activity is transduced by RAS/MEK/ERK, JAK/STAT, and phospholipase C-γ pathways.[27] These signalling pathways may also play critical roles in the activation of primordial oocytes. Hence, the activation of PI3K alone does not replace KIT activity. Other pathways may indeed contribute to the repression of PTEN activity within oocytes.

INTRACELLULAR PHOSPHATIDYLINOSITOL (3,4,5)-TRISPHOSPHATE LEVELS ARE THE CRITICAL DETERMINANT OF OOCYTE SURVIVAL

The activation of *Pik3ca** in primordial oocytes by *Gdf9-iCre* enlarged ovaries by retaining a higher number of follicles per ovary, including PF. This was unexpected, because the enlargement of ovaries in mutant mice of PI3K-mTOR negative regulators is due to synchronous activation of all PF, whereas the ovaries of *oo-Pik3ca** mice contained the same number of PF as wild-type littermates at PD28. In female mice, apoptosis normally eliminates ~80% of germ cells between meiotic entry and germ cell nest breakdown during embryogenesis.[2] The apoptotic elimination of oocytes continues even after formation of PF during the first week of postnatal development. This naturally occurring selective-loss of oocytes is under the control of the TAp63α transcription factor encoded by *Trp63*; when *Trp63* is conditionally ablated in oocytes by *Gdf9-iCre*, the number of PF per ovary at PD5 is doubled compared to normal ovaries.[28] PF number in neonatal ovaries was also increased in null mutant mice of Bcl-2-associated X protein,[29] phorbol-12-myristate-13-acetate-induced protein 1,[30] p53 upregulated modulator of apoptosis,[30,31] Bcl-2-like protein 11, and Bcl-2-modifying factor,[30] the proapoptotic transcriptional targets of TAp63α in oocytes. The *oo-Pik3ca** mice were defective in TAp63α-regulated selective-loss of oocytes in neonatal ovaries, and the apoptotic markers, such as cleaved poly (ADP-ribose) polymerase and Bcl-2-associated X protein, were significantly attenuated. The phenotypes of *oo-Pik3ca** mice mimic those of mice defective in

TAp63α-regulated oocyte apoptosis, and their follicle number at PD5 was twice that of normal ovaries.[24] Hence, increase in PIP3 blocks the TAp63α-regulated oocyte apoptosis during neonatal development.

Although the inhibition of TAp63α-regulated apoptosis by PIP3 was unexpected, the PI3K/AKT/mTOR signalling pathway is known to play a key role in the survival of oocytes in follicles of all stages (Figure 1 and 2). For instance, oocyte-specific PDK1 null mice progressively lose ovarian follicles at any stage; however, the loss of oocytes in *Pdk1* cKO mice was delayed by conditional deletion of *Pten* in the oocyte.[33] Furthermore, an oocyte-specific null mutation in ribosomal protein S6 (*RpS6*), a downstream target of the PI3K/AKT/mTORC1/rpS6 kinase beta-1 signalling pathway (Figure 1), gradually lost ovarian follicles.[33] Paradoxically, mTORC1 activity is dispensable for the normal folliculogenesis;[34] the fertility remained normal in oocyte-specific deletion of *Rptor* gene encoding the regulatory-associated protein of mTOR (RAPTOR),[35] which is essential for the assembly of mTORC1.[36] Similarly, the ovaries of *Pdk1* cKO mice and *RpS6* cKO mice also contained growing follicles. The dispensability of key positive signalling molecules in the PI3K/AKT/mTOR pathway suggests the functional redundancies between the positive regulators. In fact, it has been suggested that loss of RAPTOR was compensated by increased PI3K.[37] These observations contrast with the essentiality of negative regulator molecules of PI3K/AKT/mTOR pathway, suggesting the dominance of 'brake' over 'accelerator' in the activation of PF.

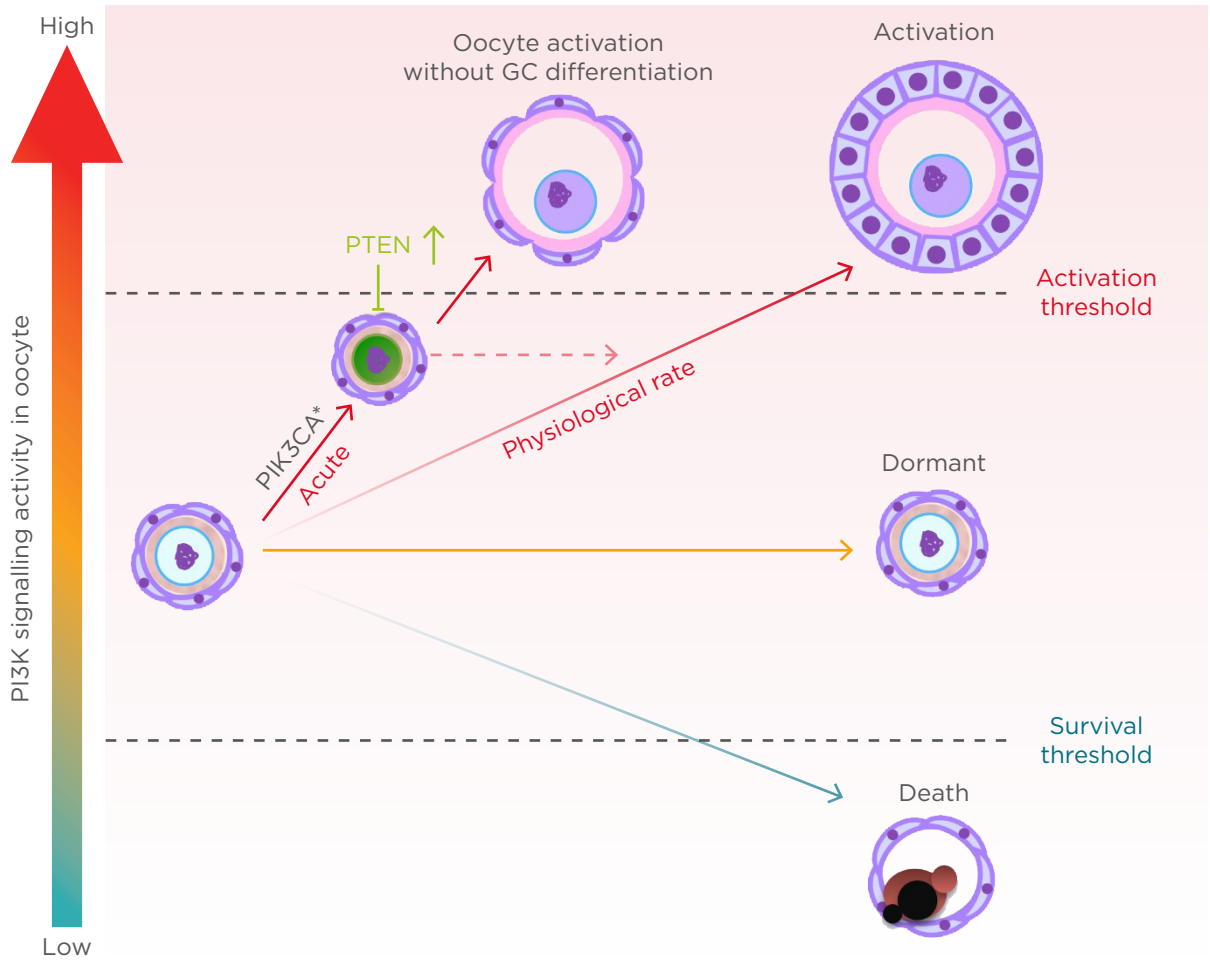

Figure 2: Oocyte phosphoinositide 3-kinase activity level and primordial follicle fate.

GC: granulosa cell; PI3K: phosphoinositide 3-kinase; PIK3CA*: constitutively active mutant PI3K; PTEN: phosphatase and tensin homologue.

CONSEQUENCES OF EXCESS PHOSPHATIDYLINOSITOL (3,4,5)-TRISPHOSPHATE IN PRIMORDIAL OOCYTES

Even though the oocyte PI3K activity and PTEN levels were elevated, the activation of PF occurred normally in the *oo-Pik3ca** mice, suggesting elevated PIP3 levels do not interfere with the innate mechanism directing PF activation (Figure 2). Meanwhile, the ovaries of *oo-Pik3ca** mice contained abnormal follicles consisting of an enlarged oocyte and pre-GC. In normal folliculogenesis, the activation of oocytes and transformation of squamous pre-GC to cuboidal GC is synchronised; however, the enlarged oocytes in the abnormal follicles were activated, as they lacked expression of FOXO3 and TAp63α in the nucleus but expressed the zona pellucida glycoprotein 1 and 3.[24] Since follicles are classified primarily by the morphology of GC, the abnormal follicle was designated as PF with activated oocytes. The absence of PF with activated oocytes in normal ovaries suggests the transformation of pre-GC to GC is a prerequisite for the activation of oocytes. Activation of KIT signalling pathways in oocytes by GC through KITL is likely involved in co-ordinating the activation of oocytes and pre-GC during normal folliculogenesis.[25] Conversely, the asynchronous activation of oocytes and pre-GC in *oo-Pik3ca** mice indicates that primordial oocytes can be activated independent of GC signalling when PI3K is constitutively active in oocytes. It has been speculated that the elevated PIP3 level allows oocytes to cross the activation threshold without stimuli from GC (Figure 2).

In the *oo-Pik3ca** mice, antral follicles with developmentally competent oocytes accumulated in the ovaries. The cumulus-oocyte complexes collected from *oo-Pik3ca** mice were capable of resuming meiosis when subjected to an *in vitro* maturation test. Despite the competence of follicles, *oo-Pik3ca** mice were anovulatory and sterile. Since ovulation occurred normally with superovulation treatment, the anovulation was not due to defective oocytes or follicles, but rather a defective endocrine system caused by an excessive number of overgrown follicles. In *oo-Pik3ca** mice, the level of follicle stimulating hormone was significantly low, while the serum concentration of both inhibin A and B were significantly increased. The altered hormones levels suggested defects in endocrine control were due to overgrown follicles.[24]

Interestingly, *oo-Pik3ca** mice had significantly larger antral follicles due to an increased proliferation rate in GC, suggesting a deregulation of growth control in GC by the expression of PIK3CA* in the oocytes. The excessive number of overgrown follicles with elevated oocyte PIP3 had an unexpected consequence in 80-day-old mice: the development of GC tumours (GCT) of the ovary. The *oo-Pik3ca** mice became cachectic by the age of 80 days due to bilateral GCT secreting activin A. The study[38] suggested that local interactions with PIK3CA*-positive oocytes during early folliculogenesis establish an activin A-mediated autocrine growth circuit programme in GCT. This explains the mechanism of GC hyperplasia in antral follicles. Meanwhile, the molecular mechanisms underlying the transformation of GC to GCT in the *oo-Pik3ca** mice are yet to be elucidated.

CONCLUSIONS

The aforementioned *oo-Pik3ca** murine model has helped elucidate the physiological roles of the balance between PI3K and PTEN within oocytes. For instance, the expression of PIK3CA* prevented the activation of TAp63α and the downstream proapoptotic signalling pathway, resulting in a higher number of surviving PF. However, it is unclear how TAp63α activity in the selective loss of PF is regulated and how PI3K activity interferes with the activation of TAp63α. In regard to the mechanism of oocyte activation, the constitutively active KIT mutant model established the sufficiency of KIT–KITL pathways to activate PF. If KIT signalling triggers the PF activation in normal folliculogenesis, the KIT–KITL pathways should be activated only in a certain number of PF in each reproductive cycle; how KIT signalling activity is controlled in the selective recruitment of PF is an intriguing question. It is also unclear if the activation of PI3K is sufficient to transduce KIT signalling in primordial oocytes. Further research is required to answer these questions.

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The Adverse Impact of Sarcopenia and Visceral Fat Deposition on the Course of Hepatocellular Carcinoma and the Role of Nutritional Interventions

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Abstract

There is mounting evidence to support the impact of sarcopenia on the prognosis of a wide range of clinical conditions. This review examines the literature on the effect of body composition measures, including sarcopenia, on outcomes in patients with hepatocellular carcinoma (HCC). Available studies support the adverse impact that sarcopenia has on overall survival, response to different treatment modalities, and tumour recurrence. Some studies have identified visceral fat deposition as a negative prognostic sign, and the incorporation of body composition measures into current HCC staging schemes have been shown to improve prognostic accuracy. On the other hand, there is a paucity of studies assessing nutritional interventions in HCC and further trials are needed to inform evidence-based practice.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of solid tumour mortality worldwide, with over half a million new cases diagnosed annually around the world.[1] In Europe, HCC accounts for around 47,000 deaths a year, with mortality rates expected to rise over the next decade.[2] The prognosis in HCC is poor, with a 5-year survival rate of approximately 10%.[3] In >90% of cases, HCC develops in patients with liver cirrhosis, most commonly due to infection with hepatitis B or C virus.[4] Other aetiological factors for the development of HCC include chronic alcohol consumption and nonalcoholic fatty liver disease (NAFLD).[4]

The Barcelona Clinic Liver Cancer (BCLC) staging system incorporates information on tumour burden, underlying liver function, and clinical features (e.g., performance status) to guide therapeutic decision-making and prognostication in HCC.[5] Treatment for HCC comprises both surgical and nonsurgical techniques. Hepatic resection or liver transplantation is reserved for early stage disease.[5] More advanced cases of HCC

are considered for nonsurgical treatment modalities, including radiofrequency ablation (RFA), transarterial chemoembolisation (TACE), and sorafenib.[5]

Although it remains one of the best HCC staging systems, BCLC has some shortcomings, particularly with regard to prognostication in the highly heterogenous patient population with intermediate-stage HCC (Stage B).[6] There is increasing research interest into the pathological, genetic, and clinical factors that may improve HCC prognostication and guide appropriate treatment choice.[7] In particular, the addition of clinical factors, such as sarcopenia and visceral fat deposition derived from computed tomography (CT) scanning, have been shown to improve prognostic accuracy compared with the BCLC staging system alone.[8] Therefore, it is important that the effect of body composition variables on outcome in HCC is delineated and the role of clinical nutrition in influencing response to surgical and medical treatments is adequately explored.

This review describes the existing knowledge on the effect of sarcopenia on outcomes in HCC and will also present the role of other body composition changes. Finally, the available studies evaluating nutritional interventions will be summarised to guide practical recommendations for optimisation of nutritional status in patients with HCC.

METHODS

Relevant studies were identified by searching the medical databases Embase and Medline (January 1996–March 2018). Additional searches were carried out using Google Scholar and Clinical Key® (Elsevier, Amsterdam, Netherlands) to identify other published data on the subject. Two separate searches were conducted; the first included the following search terms: "Hepatocelluar carcinoma" AND ("Sarcopenia" OR "Body composition"). The second search included the following terms: "Hepatocellular carcinoma" AND ("Nutrition support" OR "Dietary supplements" OR "Enteral nutrition" OR "Nutrition intervention"). A total of 139 and 209 studies were generated from the two searches, respectively, and relevant articles were selected following screening of titles, keywords, and abstracts.

All studies, either interventional or observational, that evaluated body composition measures or nutritional interventions in HCC were included. Studies evaluating sarcopenia were required to quantify skeletal muscle mass by CT or magnetic resonance imaging (MRI) scanning, which are considered the gold standard techniques for estimating muscle mass.[9] These methods overcome the difficulties associated with accurate nutritional assessment in patients with ascites and oedema related to underlying liver disease. Eighteen papers were selected for inclusion in this review; Tables 1, 2, and 3 summarise the chosen studies, including study design, participants, and findings. Excluded articles were those with no relevance to the topic, general review articles, and conference abstracts.

RESULTS

The Effect of Sarcopenia on Hepatocellular Carcinoma

Sarcopenia is associated with ageing and chronic illness and is defined by the European Working Group on Sarcopenia in Older People (EWGSOP) as the loss of muscle mass and strength.[9] Most of the available studies on sarcopenia and HCC, however, are retrospective and did not include measurements for muscle strength at the data collection stage. Therefore, for the purposes of this review, sarcopenia is used interchangeably with the skeletal muscle index or the cross-sectional area of muscles at the third lumbar vertebral level on CT scanning, measured in cm^2/m^2.

Meza-Junco et al.[10] found that sarcopenia was an independent risk factor for mortality in 116 patients with HCC undergoing evaluation for liver transplantation (hazard ratio [HR]: 2.04; p=0.02). Later, Iritani et al.[11] evaluated 217 treatment-naïve HCC patients of any stage and found that those with sarcopenia had a significantly reduced overall survival (p=0.0043). In 2017, two further retrospective analyses[12,13] identified sarcopenia as a significant risk factor for overall survival in 92 and 178 newly diagnosed HCC patients, respectively. A further study in 2017[14] showed that patients with sarcopenia died significantly earlier than nonsarcopenic patients (p=0.007); however, this difference was

not measured following adjustment for age, sex, tumour size, tumour number, and the degree of portal vein invasion.

Two recent meta-analyses[15,16] corroborated the above findings. Zhang et al.[15] evaluated eight retrospective studies that included 1,161 patients undergoing mostly surgical interventions or curative RFA. They showed that HCC patients with sarcopenia had a poorer prognosis with respect to the 1-year and 3-year overall survival, 5-year disease-free survival, incidence of recurrence, and post-treatment overall complications. Chang et al.[16] investigated 13 studies comprising 3,111 patients with HCC of varying stages. A significant association between sarcopenia and all-cause mortality was demonstrated (HR: 1.95; 95% confidence interval: 1.60–2.37). Similarly, loss of skeletal muscle mass was associated with tumour recurrence (adjusted HR: 1.76; 95% confidence interval: 1.27–2.45). In both analyses, the researchers cited the limitations of the included studies, including the absence of a consistent definition for sarcopenia in terms of cm^2/m^2, low patient numbers, and a lack of any measures for muscle function.

Table 1: Summary of studies assessing the impact of sarcopenia on hepatocellular carcinoma.

Study	Study design	Participants	Study focus	Significant findings
Meza-Junco et al.,[10] 2013	Retrospective cohort study.	116 patients with HCC being evaluated for liver transplant.	To determine the link between sarcopenia and mortality.	Sarcopenia was a strong and independent risk factor for mortality (HR: 2.04; p=0.02).
Iritani et al.,[11] 2015	Retrospective cohort study.	217 treatment-naïve patients with HCC.	To determine whether skeletal muscle depletion is a significant prognostic factor for HCC.	Sarcopenic patients showed significantly lower overall survival than those without sarcopenia (p=0.0043).
Begini et al.,[12] 2017	Retrospective cohort study.	92 consecutive HCC patients.	To assess the influence of sarcopenia on overall survival in cirrhotic patients with HCC.	Mean OS reduced in sarcopenic patients: 66 weeks versus 123 weeks (p=0.003).
Ha et al.,[13] 2017	Retrospective cohort study.	178 patients with newly diagnosed HCC.	To demonstrate the prognostic significance of changes in body composition in patients with newly diagnosed HCC.	The presence of sarcopenia at HCC diagnosis was independently associated with survival (p=0.04).
Imai et al.,[14] 2017	Retrospective cohort study.	351 patients with HCC.	To identify factors that contribute to skeletal muscle depletion in HCC patients.	Sarcopenia patients died significantly earlier than non-sarcopenia patients (p=0.007).
Zhang et al.,[15] 2017	Systematic review and meta-analysis.	1,161 patients undergoing surgical resection or curative ablation.	To evaluate the clinical significance of sarcopenia in the treatment of patients with primary liver tumours.	There was a significant difference between patients with or without sarcopenia in 1-year and 3-year OS (p=0.0004; p=0.03, respectively). There was no significant difference in 5-year OS (p=0.08). Sarcopenia had a significant impact on recurrence (p=0.002).
Chang et al.,[16] 2018	Systematic review and meta-analysis.	3,111 HCC patients at varying stages.	To assess the link between sarcopenia and outcomes.	A significant association between sarcopenia and all-cause mortality was demonstrated (HR: 1.95; 95% CI: 1.60–2.37). Loss of skeletal muscle mass associated with tumour recurrence (adjusted HR: 1.76; 95% CI: 1.27–2.45).

CI: confidence interval; HCC: hepatocellular carcinoma; HR: hazard ratio; OS: overall survival.

Table 2: Summary of studies evaluating the effect of adipose tissue distribution on hepatocellular carcinoma.

Study	Study design	Participants	Study focus	Significant findings
Fujiwara et al.,[19] 2015	Retrospective cohort study.	1,257 patients with HCC.	To analyse the impact of body composition (BMI, skeletal muscle mass, intramuscular fat deposition, IMF, VSR) on prognosis in HCC.	Sarcopenia, IMF, and VSR were significant predictors of mortality in HCC (HR: 1.52, 1.34, 1.35, respectively). BMI was not.
Montano-Loza et al.,[20] 2018	Prospective cohort.	247 patients who underwent liver transplantation, 96 of whom had HCC.	To determine whether VATI was associated with HCC recurrence following liver transplantation.	In male patients with HCC who underwent liver transplant, a VATI of ≥65 cm^2/m^2 was independently associated with higher risk of HCC recurrence (HR: 5.34; 95% CI: 1.19–23.97; p=0.03).
Saito et al.,[21] 2015	Retrospective cohort study.	96 consecutive HCC patients who underwent TACE.	To identify independent factors to predict overall survival of HCC patients after TACE.	Multivariate analyses showed visceral fat accumulation was an independent factor associated with 1-year mortality after TACE (p=0.033).
Parikh et al.,[22] 2018	Retrospective cohort study.	165 patients in total; 75 patients in derivation cohort; 90 patients in validation cohort.	To determine if body composition could predict survival among patients undergoing TACE.	Visceral fat density was the only factor predictive of overall and 1-year survival (p<0.001). Patients with higher visceral fat density were more likely to experience hepatic decompensation.

CI: confidence interval; HCC: hepatocellular carcinoma; HR: hazard ratio; IMF: intramuscular fat deposition; TACE: transarterial chemoembolisation; VATI: visceral adipose tissue index; VSR: visceral-to-subcutaneous adipose tissue area ratio.

Overall, the available studies demonstrate the adverse impact that sarcopenia has on the prognosis of HCC of varying stages, both prior to and following medical and surgical treatments. Sarcopenia also increases the risk of tumour recurrence following curative treatment. However, the studies are hampered by their mainly retrospective designs, and further prospective studies incorporating measurements of muscle strength and physical function are needed to determine whether these better predict the outcome than use of muscle mass alone. Finally, the available research does not address the underlying mechanisms by which sarcopenia worsens outcomes in HCC. An increased understanding of these mechanisms of muscle depletion would engender improved interventions and treatment strategies.

The Effect of Adipose Tissue Distribution on Hepatocellular Carcinoma

NAFLD has more recently been recognised as another important aetiological factor for the development of liver cirrhosis and HCC.[17] Liver cirrhosis in this context can lead to further skeletal muscle loss and gain of adipose tissue, culminating in sarcopenic obesity.[18] NAFLD is also one of the main risk factors for HCC development in the absence of established liver cirrhosis.[17]

A handful of studies have demonstrated the importance of adipose tissue distribution on outcomes in HCC. Fujiwara et al.[19] examined the CT scans of 1,257 patients with HCC of differing stages and with differing treatment plans. Multivariate analysis revealed that, in addition to low skeletal muscle index, both high visceral-

to-subcutaneous adiposity ratio and low mean muscle attenuation (increased intramuscular fat deposition) were significantly associated with mortality (HR: 1.52, p=0.001; HR: 1.35, p=0.005; and HR: 1.34, p=0.02, respectively). In liver transplantation, one study found that male patients with cirrhosis and increased visceral adiposity had higher rates of HCC recurrence.[20] Similarly, two studies[21,22] evaluating body composition measures in HCC patients undergoing TACE found that patients with higher visceral fat density and area were at an increased risk of death at 1 year. Moreover, patients with higher visceral fat density were more likely to experience hepatic decompensation after TACE (p<0.001).

Table 3: Summary of studies evaluating nutritional or exercise interventions in hepatocellular carcinoma.

Study	Study design	Participants	Study focus	Significant findings
Fan et al.,[27] 1994	Prospective study.	124 patients undergoing resection of HCC; 64 patients in intervention group; 60 patients in control group.	To investigate whether perioperative nutritional support could improve outcomes in patients undergoing hepatectomy for HCC.	Reduction in the overall postoperative morbidity rate in the perioperative-nutrition group.
Ziegler,[28] 1996	Prospective, randomised, controlled trial.	150 consecutive patients with resectable HCC were randomly assigned to receive either perioperative PN in addition to a usual oral diet, or to no additional therapy.	To evaluate the efficacy of perioperative PN in patients requiring hepatectomy for primary HCC.	No significant difference in postoperative hospital mortality occurred between groups (PN 8% versus control 15%; p=0.30). Perioperative PN use was associated with a significant reduction in overall postoperative morbidity rate (PN 34% versus control 55%; p=0.02).
Bothe and Steele,[29] 1997	Prospective study.	124 patients undergoing resection of HCC. 64 patients were randomly assigned to receive perioperative IV nutritional support in addition to an oral diet, and 60 patients were randomly assigned to a control group.	To investigate whether perioperative nutritional support could improve outcome in patients undergoing hepatectomy for HCC.	Reduction in the overall postoperative morbidity rate in the perioperative-nutrition group as compared with the control group.
Yao et al.,[30] 2015	Prospective cohort study.	43 patients with and 36 patients without preoperative nutrition.	To compare short-term outcomes between HCC patients with and without preoperative nutrition.	Preoperative enteral nutrition group had shorter lengths of postoperative hospital stays (p=0.007), less exogenous albumin infusion (p=0.030), earlier first exhaust time (p=0.043), and first defection time (p=0.001) observed. No significant differences observed in incidence of overall complications, infectious complications, or major complications.
Nishikawa et al.,[33] 2012	Prospective cohort study.	99 patients who underwent TACE for HCC.	To examine the significance of BCAA treatment before TACE for HCC.	Serum albumin level and Child–Pugh score improved significantly in the BCAA group as compared with the control group after TACE (p<0.05).

Table 3 continued.

Study	Study design	Participants	Study focus	Significant findings
Morihara et al.,[34] 2012	Prospective study.	30 patients who had RFA for HCC randomly assigned to a standard diet group (control), a morning BCAA administration group, or a LES with BCAA administration group.	To examine whether BCAA-enriched nutrient mixture as a LES helps maintain and/or improve liver functioning in liver cancer patients who have undergone RFA for HCC.	Compared to the control and morning BCAA groups, the LES with BCAA group experienced a rapid and significant improvement in albumin and total serum bilirubin levels.
Chen et al.,[35] 2015	Meta-analysis.	11 eligible studies (974 patients in total) were evaluated and included in the analysis.	To assess supplementation effects of oral BCAA in HCC patients undergoing interventions.	Oral BCAA supplementation helped maintain liver reserve with higher serum albumin (p=0.022) and lower rates of ascites (p=0.029) and oedema (p=0.035) than the control group.

BCAA: branched-chain amino acid; HCC: hepatocellular carcinoma; IV: intravenous; LES: late evening snack; PN: parenteral nutrition; RFA: radiofrequency ablation; TACE: transarterial chemoembolisation.

The reasons for the negative impact of visceral and intramuscular adipose tissue deposition on HCC outcomes are not fully understood. In sarcopenic obesity, muscle depletion is characterised by both a reduction in muscle size and an increased proportion of inter and intramuscular fat deposition, named myosteatosis.[23] Myosteatosis is associated with increasing insulin resistance and decreased strength and mobility.[24] Visceral fat accumulation increases the levels of proinflammatory adipokines, such as tumour necrosis factor-alpha, interleukin-6, and monocyte chemoattractant protein-1, and decreases the level of the anti-inflammatory adipokine adiponectin.[25] Excess visceral fat may also contribute to portal hypertension because free fatty acids and adipokines released from visceral fat flow directly into the liver via the portal vein.[26] It is clear the exact mechanisms underlying the interaction of visceral adiposity, liver function, and prognosis in HCC are highly complex and require further investigation.

Nutritional Interventions to Improve Outcome in Hepatocellular Carcinoma

Interventional studies addressing the effect of nutritional therapy in HCC patients are few. The benefits of perioperative nutrition support on outcomes were evaluated in a series of studies in the mid-1990s.[27-29] These demonstrated that perioperative nutrition support for 14 days with a parenteral nutrition (PN) solution rich in branched-chain amino acids (BCAA) resulted in a reduction of morbidity, diuretic use for ascites control, weight loss post-hepatectomy, and liver function, as measured by indocyanine green clearance. A more recent cohort study[30] compared patients who had received enteral nutrition 3 days prior to hepatic resection with a medium-chain triglyceride-rich formula with those who had not. They found a shorter length of hospital stay and less exogenous albumin use in the group that had received enteral nutrition. There were no significant differences seen in the incidence of overall complications, infectious complications, or major complications. Additional data are needed to delineate further the relationship between pre and postoperative nutrition support and outcomes following HCC resection.

The benefits of BCAA supplementation and a late evening snack in patients with liver cirrhosis alone have been explored recently, with studies suggesting that these strategies hold promise in reversing anabolic resistance and sarcopenia, improving quality of life.[31,32] In HCC, one study[33] evaluated the effects of

BCAA supplementation in 40 patients 1 month prior to TACE and compared these to 59 control patients on a normal diet. They found that the BCAA group had significantly improved albumin levels and Child–Pugh scores at 3 and 6 months post-TACE ($p<0.05$). Similarly, the benefits that a late evening snack containing BCAA had in improving Child-Pugh scores were demonstrated by Morihara et al.[34] Results from a meta-analysis of 11 studies[35] showed that BCAA supplementation conferred a 3-year mortality benefit, higher serum albumin levels, and lower rates of ascites. The 1-year mortality and HCC recurrence rates were not improved by BCAA supplementation, however, and many of the included studies were retrospective and had small sample sizes. All participants in this study had liver cirrhosis and therefore the benefit of BCAA supplements in HCC patients without cirrhosis is unknown.

DISCUSSION

There is much evidence to support the deleterious effects of sarcopenia on a wide range of clinical conditions. This is no different in HCC and the available studies demonstrate the adverse impact of sarcopenia on overall survival, response to different treatment modalities, and tumour recurrence following curative resection. In addition to sarcopenia, some studies have identified visceral fat deposition as a negative prognostic sign and the development of sarcopenic obesity appears to drive poorer responses to treatment and reduces survival rates.

Nonetheless, the available body composition data in HCC are limited to mainly retrospective studies, making conclusions on causality difficult. For example, it is unclear whether sarcopenia is driven by the catabolic effects of liver cirrhosis, neoplasia, or a combination of the two. Increasingly, HCC in NAFLD[23] occurs in the absence of cirrhosis, and the mechanisms by which intrahepatic, visceral, and intramuscular fat deposition causes neoplastic effects are yet to be established. An increased understanding of these mechanisms would help inform practical evidence-based guidance on nutrition support in HCC, and this review has highlighted the distinct paucity of nutrition intervention studies in this field. Inferences on the optimum nutrition management from guidance on liver cirrhosis and cancers may not be applicable to HCC patients given the unique cell biology of the condition and complex interplay with liver function and gut microbiota.

Future studies, therefore, should focus on the mechanisms by which sarcopenia and adipose tissue distribution effect HCC prognosis. Whether these measures adversely impact survival by undermining liver function or promoting tumour invasion and metastasis is unknown. An improved understanding of the relationship between body composition, response to therapy, and prognosis will enable the formulation of a tailored management strategy. Nutritional intervention studies in HCC are warranted to investigate the effect of manipulating body composition on outcomes. Although notoriously limited due to the complexity of modulating dietary intake, such studies should prospectively evaluate optimum dietary composition, the effect of BCAA supplementation, enteral tube feeding, vitamin D repletion, and the impact of exercise and physiotherapy.

In terms of current practical recommendations, clinicians should identify HCC patients at risk of sarcopenia at an early stage in their pathway and during follow-up appointments. Full anthropometric measures should be taken at regular intervals, including subjective global assessment and grip strength. BMI should not be used as a marker of malnutrition in such patients.[12,13,36] Where available, the analysis of psoas muscle cross-sectional area at the initial staging CT scan would more accurately identify those with sarcopenia. Alternatively, the bioelectrical impedance measure phase angle has been shown to correlate with survival in HCC and can be measured at the bedside.[36] Patients with underlying liver cirrhosis should receive adequate calorific intake as dictated by well-recognised international guidelines (for example, the European Society for Parenteral and Enteral Nutrition [ESPEN] guidance[37]). Patients with cirrhosis should also be considered for BCAA supplementation and the importance of a late evening snack should be re-enforced. Nutritional support in the form of oral nutritional supplements or nasogastric feeding should be administered if the patient is unable to meet their nutritional requirements. Patients being

considered for hepatectomy should receive intensive preoperative dietary input and have a low threshold for preoperative enteral feeding. Finally, it goes without saying that all patients should receive regular dietary follow-up and have their nutritional statuses re-evaluated when a change in medical or surgical therapy is being considered.

CONCLUSION

To conclude, the available literature supports the adverse impact of sarcopenia and visceral fat deposition on overall survival, response to different treatment modalities, and tumour recurrence in HCC. There is a lack of studies investigating the optimum nutritional support for this condition and it is imperative that this field grows to complement future advances in medical therapy.

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What's New

Striking a Balance Between Vitamin D Levels and Sun Exposure

UNCERTAINTY regarding how to strike a balance between ensuring children receive enough sun exposure to produce healthy levels of vitamin D and guarding them from the harmful effects of too much ultraviolet (UV) light has been tackled by a study conducted by researchers from King's College London, London, UK in collaboration with researchers in Poland and Denmark. Following their study, the researchers urge that even more caution should be taken when protecting children from the harmful effects of the sun. With the European summer fast approaching, this is a timely finding and recommendation.

"Our study suggests that only small amounts of exposure to the sun are needed to ensure vitamin D sufficiency, so we should make sure that children always have ample sun protection when playing outside for long periods."

The senior author of the study, Prof Antony Young, King's College London, explained the motivation behind the investigation: "Many parents are already very careful about protecting their children from the harmful impact of the sun, but it can be a confusing message when trying to balance this with the need for children to be healthy, exercise, play outside, and produce sufficient levels of vitamin D." To address this issue, the researchers studied the impact of a 12-day beach holiday on the Baltic coast on the levels of cyclobutene pyrimidine dimers (CPD), biomarkers of DNA damage that can lead to skin cancer, and serum 1,25-dihydroxyvitamin D ($25(OH)D_3$) in 32 Polish children.

The researchers collected blood and urine samples from each of the children both prior to and after the holiday and then analysed these samples to determine the levels of CPD and $25(OH)D_3$. UV exposure was determined by an electronic device worn on the wrist; furthermore, the children recorded information about sunbathing, sunburn, and sunscreen use in diaries.

The results showed that, compared to baseline levels at the beginning of the holiday, there was an average increase of 25% in vitamin D levels in the blood and an almost 13-fold increase in CPD numbers by the end of the holiday. These results suggest that children may be more sensitive to the damage caused by overexposure to UV than previously thought, or that they are better at repairing this type of damage. Prof Young concluded: "Our study suggests that only small amounts of exposure to the sun are needed to ensure vitamin D sufficiency, so we should make sure that children always have ample sun protection when playing outside for long periods."

JAK Inhibitors and Alopecia

RESEARCHERS from Columbia University, New York City, New York, USA, have demonstrated the effectiveness of ruxolitinib for the treatment of alopecia areata (AA). Of the 12 patients involved in the open-label, uncontrolled, pilot study, 9 showed a ≥50% improvement in Severity of Alopecia Tool (SALT) scoring.

There have been a number of publications highlighting the potential role JAK inhibitors could have in treating AA. Building on previous studies, the researchers investigated whether ruxolitinib, a U.S. Food and Drug Administration (FDA)-approved JAK inhibitor for the treatment of polycythemia vera and myelofibrosis, could be an effective treatment of AA.

The trial included 12 patients with moderate-to-severe AA; 75% of patients improved by ≥50% in SALT scoring after receiving 20 mg ruxolitinib twice daily for 3–6 months. Responses were beginning to present within the first month of treatment, and 11 patients achieved ≥50% hair regrowth by Week 12. By the end of treatment, 7 of the 9 responders achieved >95% regrowth; the 2 patients who did not achieve 95% regrowth achieved 85% and 55% regrowth, respectively. No serious adverse effects were experienced and no patients had to withdraw from treatment.

Once the trial was complete, patients were followed-up to assess the durability of the response once ruxolitinib treatment was stopped. Three weeks after treatment cessation, 30% of patients began shedding hair, and substantial hair loss was reported 12 weeks post ruxolitinib treatment.

Researchers also conducted gene expression profiling and identified that nonresponders to treatment had lower expression of genes encoding for proteins that mediate Type I cellular immunity and drive the pathogenesis of AA.

These findings are particularly poignant considering the lack of effective treatment currently available for alopecia, despite the global lifetime incidence estimate of 2%. Another JAK inhibitor, tofacitinib, was also investigated in 12 patients with moderate-to-severe patchy AA, alopecia totalis, or alopecia universalis. These results indicate that JAK inhibitors are a viable alopecia treatment method; however, further investigation is still warranted.

These results indicate that JAK inhibitors are a viable alopecia treatment method; however, further investigation is still warranted.

What's New

Genetic Variation Determines Survival After Blood and Marrow Transplant

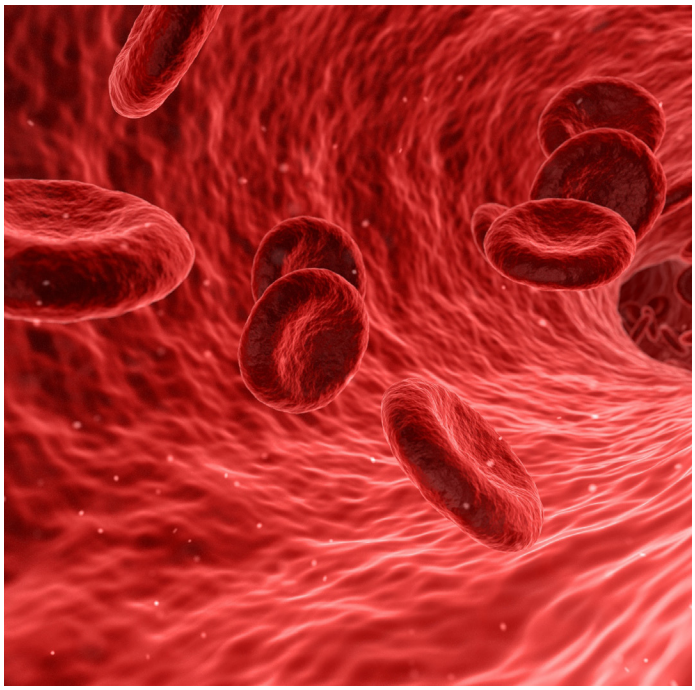
SURVIVAL rates after blood and marrow transplant (BMT) have been shown to correlate with the presence of rare variations in specific genes of unrelated blood or marrow donors. BMT is known to be both life-saving and potentially life-threatening, so these study results may be of great significance for improving individual risk prediction and prognosis for patients undergoing the procedure.

As part of a collaboration between Roswell Park Comprehensive Cancer Center, Buffalo, New York, USA and The Ohio State University, Columbus, Ohio, USA, researchers genotyped the coding regions of the genomes of approximately 2,500 acute leukaemia patients and their human leukocyte antigen (HLA)-matched unrelated blood or marrow donors. The team found that variation in several novel genes significantly impacted the survival of BMT patients; for example, when the genotype of the *TEX38* gene differed between recipients and donors, the risk of transplant-related death was increased. This trend was more significant in female recipients or donors and was attributed to a change in the TEX38 antigen, increasing the affinity and binding strength to major histocompatibility complex (MHC) Class I molecules, shown to be important in BMT outcomes.

"We are hoping that additional studies of this type will continue to discover novel genes leading to improved outcomes for patients."

The team also noted that patients who received blood or marrow from donors with rare variation in *NT5E* had a significantly lower risk of death from leukaemia after BMT due to reduced activity of the enzyme. These results were consistent with previous findings that have showed blocking the *NT5E*-encoded enzyme can reduce the rate of tumour growth.

As the first study to analyse the relationship between rare genetic variation and survival rates after BMT from an HLA-matched unrelated donor, these results shed light on areas that have not previously been explored. Co-first author of the study, Dr Qianqian Zhu, Roswell Park Comprehensive Cancer Center, commented on the future implications of the study: "We are hoping that additional studies of this type will continue to discover novel genes leading to improved outcomes for patients." As well as improving prognosis for BMT patients, the team hope that identifying genetic variation will help optimise donor selection in the future.



Idiopathic Pulmonary Fibrosis: Targeting Prevention

IDIOPATHIC pulmonary fibrosis (IPF) may one day be a preventable disease as scientists learn how to block the effects of a gene that causes fibrosis, according to new research from Michigan Medicine, Ann Arbour, Michigan, USA.

IPF causes difficulty in breathing due to fibrosis, or tissue scarring, present in the lungs. The disease often leads to respiratory failure in the patient, resulting in a life expectancy of 3–5 years. Despite the severity of this disease, general awareness is low. The U.S. Food and Drug Administration (FDA) approved the first drugs for use in IPF patients 3 years ago; however, these drugs are only able to slow disease progression and do not rectify the fibrosis that is already present.

Previous research studies have shown that the *FOXM1* gene is responsible for initiating cancer cell growth in humans. The research team questioned whether blocking this gene could result in the prevention or slowing of fibroblast development, the cells that are responsible for the scar tissue production seen in fibrotic lung diseases.

To investigate this hypothesis, mice were given a toxic drug that triggered lung fibrosis, and were then examined along with fibroblasts from the lungs of patients with IPF. It was found that both the mice and patient fibroblasts had increased expression of the gene. The researchers then genetically engineered the mice to eliminate the *Foxm1* gene. When administered with the same toxic drug, it was found that the mice lacking *Foxm1* did not develop fibrosis.

The team then took the study one step further, attempting to block the *Foxm1* gene in those mice that had not been genetically engineered. They used a drug called siomycin, an experimental compound that has been designed to block *FOXM1* in humans. When using siomycin, the mice did not develop further fibrosis, and the amount of scar proteins produced was significantly reduced. Prof Marc Peters-Golden, Division of Pulmonary and Critical Care Medicine, Michigan Medicine, concluded: "We proved that, in principle, if we block *FOXM1*, we can reduce the activation of fibroblasts as well as the process of fibrosis itself."

Although siomycin has not yet been approved for human use, the researchers are hopeful for the treatment's future. Further drugs are in development that, once demonstrated to be safe in humans, will move forward to clinical trials.

"We proved that, in principle, if we block FOXM1, we can reduce the activation of fibroblasts as well as the process of fibrosis itself."

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