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+ DERMATOLOGY

After Conventional Wisdom Has Failed, What Drives Wound Healing?

+ RESPIRATORY

Immunotherapy in Small Cell Lung Cancer

+ ONCOLOGY

A New Twist to Ibuprofen

THE EUROPEAN MEDICAL JOURNAL

+ EDITOR'S PICK

Sex Differences in Paediatric and Adult Asthma

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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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EMJ 4.2

European Medical Journal 4.1

Whether you are a fledgling physician looking for an update or a veteran scientist looking for inspiration for your next research project, this journal has something for everyone.

VIEW JOURNAL ←

Welcome

The sharing of concepts, collaboration, and the cross-pollination of ideas are important mechanisms in driving innovation and creativity, and the medical field is no exception. Our flagship journal has been curated to provide the vehicle for this to take place. By including articles from across the therapeutic spectrum, we hope to create fertile ground for the flowering of fresh ideas that will advance scientific knowledge and patient care.

"The sharing of concepts, collaboration, and the cross-pollination of ideas are important mechanisms in driving innovation and creativity..."

Ibuprofen is familiar to a vast swathe of the general public, who tend to be acquainted with its role in over-the-counter pain relief. Jordan et al. invite the reader to consider this ubiquitous drug from a different perspective: as a chemoprotective tool. The authors particularly focus on its action in alternative splicing. Much like ibuprofen, the general public is also well aware of obesity. This condition has been linked with a myriad of healthcare issues, including cancer and cardiovascular disease. Katarzyna Rygiel carefully examines the relationships between breast cancer, obesity, and cardiovascular diseases, and additionally highlights how to develop a weight management programme to assist in the best management of patients.

Also in the field of cancer, we have included an article by the Editor-in-Chief of *EMJ Respiratory*, who has thoroughly explored the current data for immunotherapy in small-cell lung cancer. Bearing in mind the disease's frequently poor prognosis, this look at a promising treatment avenue is exceedingly timely. Unfortunately, the constraints of the page prevent me from highlighting each article as it deserves, so I will limit myself to the fact that *EMJ 4.2* also contains papers from the fields of diabetes, respiratory medicine, dermatology, and reproductive health.

Finally, I would like to extend my thanks to all of you who made this edition of EMJ possible. With your diligent assistance, we have once again been able to produce a journal that I am proud to share with the medical community. I urge everyone, whatever your speciality, to take some time to read what lies inside. What you read today may reverberate with you and shape your practice for years to come.

Spencer Gore

Spencer Gore

Chief Executive Officer, European Medical Group

IN YOUR PATIENTS WITH SEVERE UNCONTROLLED ASTHMA

LOOK BEYOND EOSINOPHIL AND IgE LEVELS IN TYPE 2 INFLAMMATION

Cytokines IL-4, IL-5 and IL-13 are key drivers of Type 2 inflammation in asthma[1-3]

1. Fulkerson P, *et al. Nat Rev Drug Discov.* 2013;12(2):1–23. **2.** Caruso M, *et al. Curr Opin Allergy Clin Immunol.* 2013;13(6):677–85. **3.** Hammad H, *et al. Nat Rev Immunol.* 2008;8:193–204.

Sanofi Genzyme and Regeneron are committed to providing resources to advance research in areas of unmet medical need among patients with inflammatory and immunologic diseases.

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SANOFI GENZYME

Foreword

Dear colleagues,

It is my great pleasure to welcome you to the second issue of the European Medical Journal of 2019, containing a carefully curated selection of articles on a myriad of fascinating and important topics. We find ourselves at an exciting time in scientific research and healthcare improvements; the contents of this journal illustrate not only the progress being made every day by clinicians and researchers, but also the great strides we have yet to make. Herein, I summarise some of the key highlights of the journal.

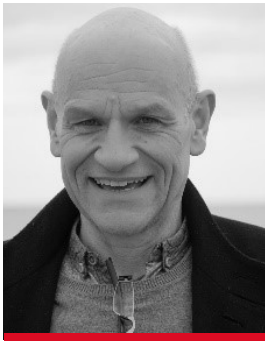
My fellow diabetologists will take great interest in the paper by Lefkovits et al. on the use of artificial pancreas in managing Type 1 diabetes mellitus in pregnancy. The improvement in glycaemic control, albeit associated with some adverse effects, provides hope to a great many people. Further improvements should pave the way to a reduction in adverse events. I am confident that research into closed loop systems, the term I prefer to artificial pancreas, will progress rapidly and find answers to some of the remaining challenges.

The importance of multi-disciplinary approaches to patient care can never be underestimated. In Rygiel's paper, the author considers the comorbidities of hormone receptor-positive breast cancer and heart disease in relation to obesity and weight reduction, offering a timely comment on the importance of lifestyle interventions focussing on weight reduction in improving outcomes for patients and survivors.

My Editor's Pick of this issue is the paper by Naeem and Silveyra on the life course of sex differences for asthma. Interestingly, they observe a 'switch' from a higher incidence rate in boys to the reverse later in adulthood when women are more likely to develop the condition. This pattern is linked to changes in hormone levels along the life course. Improved understanding of these developments will create new options for asthma treatment strategies.

I would like to thank all of the contributors to this issue for their hard work and dedication to the dissemination of scientific research. I would also like to take this opportunity to thank our readers; although I have highlighted here some of my particular comments, please contribute to the discussion by getting in touch to give us your feedback on the journal and your opinion on the topics covered.

Kind Regards,



Jörg Huber
University of Brighton, UK

The IBD Pathway: From a Patient Perspective

This symposium took place on 8th March 2019 as part of the 14th European Crohn's and Colitis Organisation (ECCO) Congress in Copenhagen, Denmark

Chairpeople: Geert D'Haens[1]

Speakers: Raja Atreya,[2] Luisa Avedano,[3] Yoram Bouhnik,[4] Maria de Jong[5]

1. University of Amsterdam, Amsterdam, Netherlands
2. University of Erlangen-Nürnberg, Erlangen, Germany
3. European Federation of Crohn's and Ulcerative Colitis Associations (EFCCA), Brussels, Belgium
4. Paris-Diderot University, Paris, France
5. Academic Medical Centre, Amsterdam, Netherlands

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This is a summary of an industry-sponsored satellite symposium at the 14th European Crohn's and Colitis Organisation (ECCO) Congress, held in Copenhagen, Denmark on 8th March 2019.

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Meeting Summary

The emergence of anti-TNF biosimilars has had significant implications for the biologic treatment of inflammatory bowel disease (IBD). Significant cost savings provide an incentive for healthcare providers to encourage the prescription of biosimilars instead of reference products. However, patients may have concerns about the switching process, the reason for the switch, or the biosimilar itself, and it is important for healthcare professionals (HCP) to take these into account to enable an informed, shared treatment decision.

The aim of this symposium was to understand treatment of IBD from the patient's perspective, especially when switching treatment to a biosimilar product. Beginning with a review of the current and future treatment landscapes, the implications of the increasing availability of biosimilars were discussed. The role of HCP in communicating information about the switch was explored by the multidisciplinary faculty who also compared switching practices at their own treatment centres and shared best practices. Alongside videos of interviews with patients who had undergone a switch to a biosimilar, a patient advocacy perspective was provided by Ms Luisa Avedano, CEO, the European Federation of Crohn's and Ulcerative Colitis Associations (EFCCA).

Introduction

A highly interactive symposium, including pre-recorded interviews with patients, was chaired by Prof Geert D'Haens. Participation by the audience was strongly encouraged through live polling and submission of questions to the faculty throughout the meeting.

In recent years, biologics have become a cornerstone in the management of IBD. As reference product patents expire, biosimilars join the treatment armamentarium, adding an exciting and relevant dimension.[1]

Overall healthcare costs are substantial in IBD, with an estimated €1,625 spent in Crohn's disease and €595 in ulcerative colitis per patient every 3 months, much of which is spent on anti-TNF agents (64% and 31% of the total cost in Crohn's disease and ulcerative colitis, respectively).[2]

Between 2007 and 2020, the introduction of biosimilars has been estimated to offer potential cost savings of between €12 and 33 billion in the European Union (EU).[3] Both direct and indirect benefits are offered by biosimilars. When biosimilars enter the market at reduced prices, this is usually accompanied by a reduction of the price of the reference product. This reduced cost burden in one product allows money to be reinvested into healthcare systems, which might enhance patients' access to effective treatments.[3-5]

While comparability between approved biosimilars and their reference products in terms of safety, efficacy, immunogenicity, and pharmacodynamics has been shown in randomised controlled trials,[6] patients may have reservations about being prescribed therapeutics other than the reference biologic or being switched to a biosimilar. A lack of confidence in treatment, driven by a lack of communication and shared, informed decision-making between the HCP and their patient in preparation for a switch, may lead to subjective loss of response or side-effects.

Multidisciplinary Approach to Managing Inflammatory Bowel Disease

Following Prof D'Haens' introduction, the roles of different members of the healthcare team in the pre-switching process were discussed by the faculty. Prof Bouhnik advised that switching should only be done based on a shared decision between the physician and their patient. During the initial switching discussion, it is essential to be clear that the proposed medication is not a new drug or mechanism of action, but is, as Prof Bouhnik noted, a: "similar: the word is important." Following this preliminary conversation, an IBD nurse would be the main point of contact. In Prof Bouhnik's opinion, physicians are often confined by the time pressures of a busy clinic,

and expert nurses are more adept at conversing with patients about switching following the initial consultation.

HCP responding to a pre-symposium questionnaire said an initial consultation to verbally discuss the switch procedure could last from 15 to >30 minutes. Additional information was provided in educational leaflets or letters, and patients were directed to educational websites. After a switch, patients were monitored and a follow-up appointment was scheduled <1 month to 3–6 months post-switch.

Ms Maria de Jong described the role of the nurse in pre-switch communication. Patients are likely to think of questions and concerns following the initial conversation with the treating physician, which they did not articulate during this consultation. It is, therefore, important to have a member of the healthcare team whom patients are able to contact readily to discuss these. The expert nurse can fill this role, with part of their time being set aside for telephone consultations with patients.

Multidisciplinary Team Communication: Healthcare Professionals

Prof D'Haens noted that the Netherlands, and, within it, the Academic Medical Centre (AMC), were among the earliest locations to adopt biosimilars, with their prescription being encouraged for economic reasons. There was, therefore, little information from other units on how best to communicate with patients about the switch process. Ms de Jong explained that a team approach was decided upon, which included gastroenterologists, IBD nurses, day care centre nurses, a PhD candidate, and the patient. Ideally, the physician talks to the patient about biosimilars in person, but occasionally a telephone consultation is required for practical reasons. Discussions last for approximately 10 minutes, during which the physician provides information and seeks informed consent for a switch to a biosimilar. The physician aims to provide relevant information regarding the switch in a positive and encouraging manner, but ultimately the patient is responsible for making an informed decision whether to undertake a switch. Ms de Jong also explained that IBD nurses at the AMC clearly explain to patients that they can switch back to the reference product after commencing the biosimilar. In addition, a flyer was developed at the AMC that could be distributed prior to the consultation to provide the patient with some context of the proposed switch or, ideally, following a consultation to provide the patient with further written information.

Prof Atreya described the initial consultation in which patients are introduced to biosimilars, stressing that the most important message to convey was: "more of trust and more of emotion"; patients successfully treated with reference biologics may be averse to changing a therapy that has improved their quality of life or even resulted in disease remission. Ms Luisa Avedano, CEO, EFCCA patient associations (PA), reported that, despite awareness of biosimilars being lower and communication strategies regarding switching being in an early stage at the time of a 2014–2015 survey, a strikingly low figure of 11.7% (n=383) of patients agreed with the statement that they: "trust their pharmacist or treating physician" if they prescribe or deliver a biosimilar following treatment with a reference product.

Prof Raja Atreya highlighted the importance of being open about economic reasons for switching from both a perspective of trust and one of practicality, noting that with sufficient numbers of patients switching to biosimilars, further HCP could be recruited to the unit, leading to a better standard of care through increased availability of staff: "They could really see the waiting times reduced and this was an important factor to motivate them," Prof Atreya explained. Prof D'Haens reported that patients may be encouraged to switch if they consider that cost savings could be used to pay for more effective treatments for other conditions where less expensive therapies are not available.

After the possibility of a switch is introduced by the physician, additional information can be provided by IBD nurses via face-to-face consultation, via telephone, or as printed material. Prof Atreya, Ms de Jong, and Ms Avedano all noted that patients are interested in the results of switching studies. "We have more information, and we can use this information," said Ms de Jong, indicating that clinical data can

be shared with, and explained to, the patient by the physician and nurse to engage with them, build trust, and ultimately make a well-informed, shared decision.

HCPs' communication with their patients can also involve directing them to other organisations. Ms Avedano iterated that EFCCA is encouraging HCP to direct newly diagnosed patients to PA. Ms de Jong agreed with the importance of this, confirming that the AMC treatment pathway included making patients aware of PA. Later in the symposium, Prof D'Haens referred to the rheumatology department at the AMC, where patients treated with reference biologics were switched to biosimilars under the care of physicians and specialist nurses, with an information package that was assembled by both HCP and representatives from PA and suggested that this may be an effective way to communicate information most appropriately to patients.

Multidisciplinary Team Communication: Patient Perspective

The first interview video saw two patients describing how and when they were first told about biosimilars. One patient, treated at AMC, was told about biosimilars and a potential switch during a routine appointment. The other patient, treated at Paris-Diderot University, Paris, France, was introduced to biosimilars at the day hospital during a reference infliximab infusion appointment, having a 20-minute conversation with his physician about a change in treatment. Initially he: "did not know what to expect." He had the opportunity to ask questions to both the physician and nurses. Following this appointment, he conducted some research on the internet to find more information and reported he: "saw that it was done in other areas of health." Discussing biosimilars with his family suggested that they had: "no more worries" than him. He found the transition to a biosimilar straightforward; both the reference product and new treatment were administered as infusions, so the change was not significant. He reported that he was: "waiting to see the effects and, in fact, there is not much of a difference."

Regarding communication between patients themselves about the switching process, Ms de Jong reported that: "patients are also talking with each other about their experience, and if it's positive it is easier." Conversations can be held at day care centres and infusion appointments, and that discussion is ongoing on social media platforms. PA, such as EFCCA, also facilitate this dialogue.

Initiating the Switch: Pathways and Programmes

Both Prof Bouhnik and Prof Atreya responded to a question from the audience asking whether they deemed any patients ineligible for a switch and both replied that, in their opinion, all patients could be initiated on a biosimilar, but that switching back is a possibility if adverse effects (AE) are subsequently experienced.

Prof Atreya reported that at his centre, the University of Erlangen-Nürnberg, Erlangen, Germany, over a 2-month period, 100% of patients with IBD on reference infliximab (N=˜200) had their treatment switched to a biosimilar. This switch was mandatory; patients not accepting the change in medication would: "have to look for another infusion centre." However, only one patient did not consent to the change, and they returned to the Erlangen-Nürnberg clinic after one infusion at another treatment centre. Notably, this patient reported that she returned primarily because she found the nursing care to be of lower quality at the new centre. Prof Bouhnik referred to a study which reported results from a French treatment centre: 86 patients with IBD receiving treatment with reference infliximab were offered a switch to biosimilar. Of these patients, 47% initially refused the switch but, of these, 78% agreed to participate in an education interview with a nurse. Following this, a total of 68% of patients finally accepted the switch.[7] These instances highlight the importance, from the patient's perspective, of the role of the nurse in the multidisciplinary team (MDT), especially in providing education in a clinical setting.

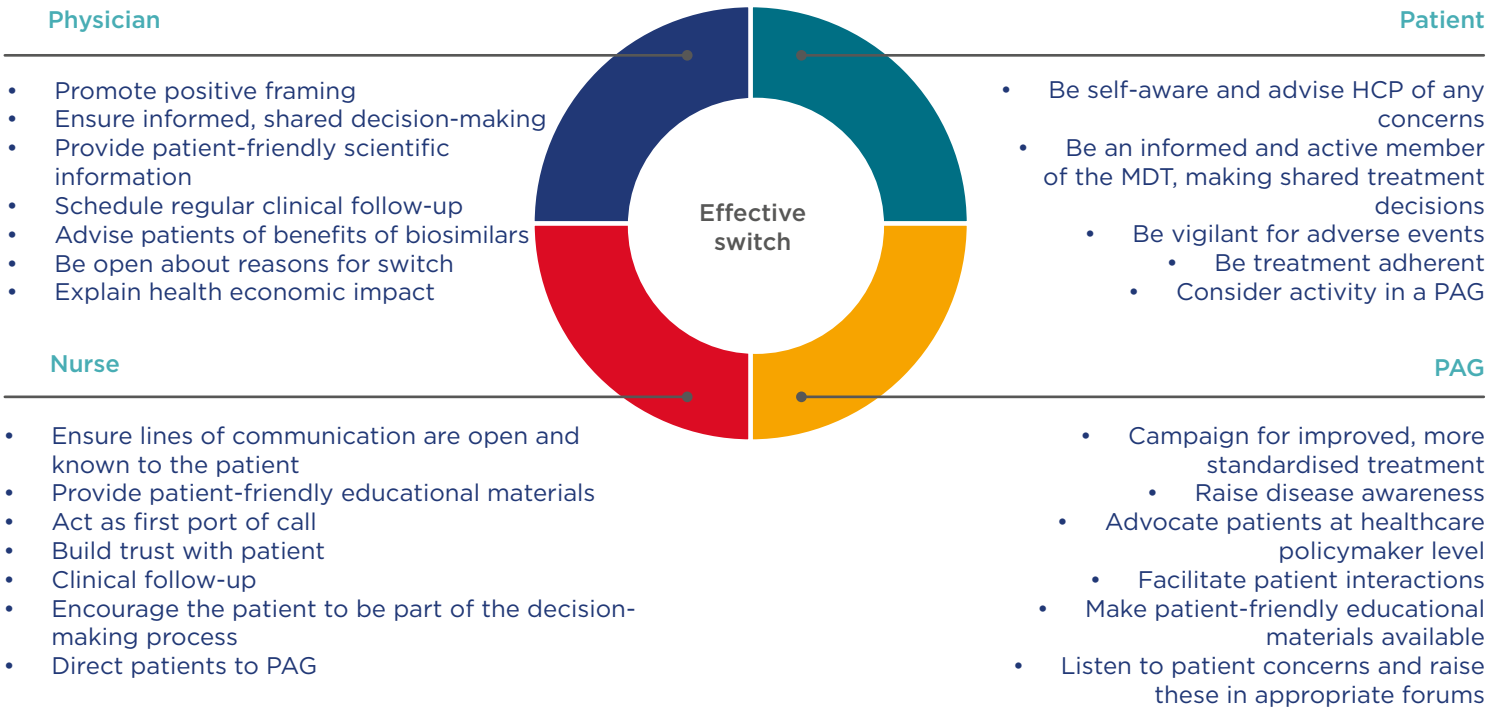

Figure 1: Roles and responsibilities of key participants for a successful switch.

HCP: healthcare professionals; MDT: multidisciplinary team; PAG: patient advocacy group.

Key Insights from Patient Associations: The Patient Representative Perspective

EFCCA represents 36 IBD PA from 35 countries and engages, on average, between 10% and 20% of patients with IBD within each country. Ms Avedano said that EFCCA was committed to "making the invisible visible" regarding IBD, and noted wide variation in switching practices between countries and, in some instances, regional differences within the same country. This variation, alongside a general lack of information about biosimilars, could make patients uncomfortable as they see: "a general picture of the situation that does not necessarily mirror reality in every country." To investigate patients' level of knowledge and perspectives about biosimilars, EFCCA developed the Biologics and Biosimilars Online Survey (BAB), which was conducted from 2014–2015.[7] Across Europe, 1,181 patients completed the survey. Just 38.0% of surveyed patients had heard of biosimilars and only 25.2% of this group of patients familiar with biosimilars reported having no concerns about them.[8]

Results of the BAB study prompted EFCCA to organise a series of advocacy and educational workshops to address the perceived lack of information available to patients.[9] These included not only patients with IBD treated with biologic therapeutics, but also patients with other immunomediated conditions who were able to offer insights into their treatment pathways and the switching process. Across disease groups, patients reported room for improvement in communication with physicians and also the crucial role of specialist nurses in treatment pathways. Between countries, there was significant variation in the availability of specialist nurses within the MDT. Prof Atreya and Prof Bouhnik said that the situation in Germany and France, respectively, was that specialist nurses were beginning to be introduced to the IBD MDT, and that the practice was growing but not currently universal. Ms Avedano reported a lack of understanding amongst policymakers with whom EFCCA engaged following these workshops to raise the concerns identified by patients, including a lack of awareness of IBD, treatment pathways, and costs (both direct and indirect) associated with the condition. Reports from the EFCCA workshops following

the BAB have suggested improvements in communication since, but there is significant scope for improvement across Europe.

Following a Switch Phase and Subsequent Follow-Up: Patient Perspective

The patient treated at AMC reported that: "the communication went well and the information provided to me was great." The patient was in close contact with the treating physician who gave guidance during and after the switch itself. Alongside this, the patient was: "always able to ask questions to the IBD nurse… which made the transition a very pleasant experience." The patient responded that he felt as good as he did before the switch and suggested that, to improve patient care following a transition to a biosimilar, communication between patients and HCP should be made as easy as possible, so that any questions and concerns can be addressed quickly. Figure 1 illustrates roles and interactions within the MDT to aid a successful switch.

Follow-Up and Faculty Advice: Optimising Post-Switch Care

Prof Bouhnik described the ongoing PERFUSE study,[10] designed to gain insights into post-switch patient perspectives by collecting data on post-switch drug survival rates. PERFUSE is a long-term, prospective, observational, multicentre cohort study investigating SB2 (an infliximab biosimilar) discontinuation in 1,500 French patients who were switched from the reference product in five autoimmune conditions. The patient perspective is also being explored via measurement of patient-reported outcomes, treatment perceptions, and satisfaction regarding the information about biosimilars that was provided to them.

Ms de Jong described a study, in which the AMC participated, that followed patients with IBD in remission for 16 weeks. Pharmacokinetics (PK) and disease activity (via the simple clinical colitis activity index or Harvey–Bradshaw index) were measured, along with antidrug antibody formation, AE, and patient-reported outcomes. In 88 patients with IBD in remission (29 at AMC) who were treated on reference infliximab for >30 weeks, a subsequent switch to a biosimilar was found to be safe and well tolerated.[11]

Prof D'Haens asked the faculty how they responded to patients who were switched to biosimilars reporting that the new product was ineffective or causing AE. Ms de Jong confirmed that the MDT would work to establish the problem, including carrying out PK investigations, such as trough levels, but that physiological reasons for inefficacy or AE may not be found. Prof D'Haens mentioned that subjective factors can be involved in patients wanting to switch back, citing the nocebo effect, and that clinical trial results and his experience indicate that a number of patients do switch back to the reference product after commencing a biosimilar.[12] Prof Bouhnik confirmed that patients had the right to switch back to reference products following a change to biosimilar treatment in France and mentioned that the nocebo effect was a noted problem, while Ms de Jong and Prof D'Haens reported a switch back rate of <5% at the AMC. Prof Atreya presented Harvey–Bradshaw index and partial Mayo scores of patients with ulcerative colitis and Crohn's disease, respectively, up to 24 weeks post-switch from reference infliximab to SB2, which showed no statistically significant difference over the study period.[13]

A New Approval: A Different Story for Adalimumab?

The experiences described so far relate to infliximab administered by intravenous infusion but, with the recent market authorisation of adalimumab biosimilars (self-administered subcutaneously), the faculty members were asked for their predictions of how the two drugs would compare in relation to switching.

Prof Bouhnik reported that there were differences in the acceptance rate of patients agreeing to switch from infliximab and adalimumab reference products to their biosimilars; in his clinical experience up to 7 out of 10 consecutive patients would not accept a biosimilar to reference adalimumab. Although real-world evidence

for the safety and effectiveness is convincing for patients, the timing of the introduction of adalimumab biosimilars means that these data have not been available until recently, accounting for this reluctance to switch. An audience member asked whether the faculty expected adalimumab switch programmes to be as simple as those for infliximab. Prof Bouhnik thought that they would be: "much more difficult… the nocebo effect will be a major problem," due to self-injection administration with a number of different devices available. Prof Atreya added that the increased logistic effort of measuring trough levels for adalimumab makes undertaking PK studies in the real world more difficult, but that experience gained in infliximab switching programmes would be valuable in facilitating the adalimumab switching procedure.

Prof D'Haens noted that, while being similar in terms of the active biologic, adalimumab biosimilars were different in terms of excipients included in the complete formulation, administration devices, stability, and shelf-life.

Furthermore, Prof D'Haens addressed the importance of biologics stability.[14] Indeed, in a study including 255 patients, only 6.7% of the patients stored all biological disease-modifying antirheumatic drug packages within the defined Summary of Product Characteristics-recommended temperature range. It was noted that SB5 (an adalimumab biosimilar) has an approved cool chain shelf life of 3 years, compared with 2 years for the other adalimumab products, and that data supporting the stability of SB5 at room temperature (25 °C) up to 28 days have been recently published (and, since the symposium, approval for storage at temperatures of up to 25 °C for a period of up to 28 days has been granted by the European Medicines Agency [EMA]),[15] which could have implications for the daily activities and lifestyles of patients.[16] Prof D'Haens noted that some preparations of adalimumab are more appropriate than others for individual patients, depending on formula, stability, and other product characteristics. Symposium attendees were asked a live polling question: "Were you aware of the differences between adalimumab products, in particular regarding stability?" and 63.3% responded that they were familiar with the subtle variations in preparations not relating to drug activity or immunogenicity.

Conclusion

The patient's perspective is paramount in the IBD treatment pathway, especially when considering switching to a biosimilar from a reference product. From the initial consultation in which the patient is introduced to biosimilars to all interactions following a switch, communication is key. Easily contactable specialist nurses in the MDT are valued greatly by patients, and advocacy groups are campaigning for increased access to MDT members, along with more consistency in messages and practices regarding biosimilars to inspire confidence in their uptake. The process of switching patients treated with adalimumab may present new challenges. Nocebo effects may have a greater impact due to the fact that these products are self-administered. It will require thoughtful communication from HCP to transfer confidence to patients, but this could lead to increased initial uptake rates and reduction of the nocebo effect.[17] With well-considered switch programmes, patients can be confident in their therapeutics with beneficial results for healthcare economics.

The importance of communication was confirmed following the symposium, when faculty members were interviewed individually and asked for advice they would offer to patients preparing for a switch. Prof D'Haens said that: "the process starts with information, but the patient may come back with questions, and then there needs to be somebody available to answer, and I think we underestimate that as physicians." Ms Avedano highlighted the importance of involving the patient in treatment decisions by providing them with educational materials appropriate to their understanding and stressed the importance of having a well-functioning MDT, including specialist nurses.

Prof Atreya said that: "you really have to be a partner," asking the patient whether, from their perspective, they noticed: "any difference in quality of life." "We can assess the clinical disease activity but what is much more important is what the patient feels," he concluded. Ms de Jong emphasised a proactive communication strategy, with the treatment centre contacting the patient, while Prof Bouhnik recommended assessing efficacy and immunogenicity of the new treatment using biomarkers or therapeutic

drug monitoring and: “especially, to give them the possibility to contact the team whenever they want.”

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The IBD Management Puzzle: Do We Have All the Pieces?

This symposium took place on the 7th March 2019 as part of the 14th congress of the European Crohn's and Colitis Organisation (ECCO) in Copenhagen, Denmark

Chairperson: Stefan Schreiber[1]

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Meeting Summary

The management of inflammatory bowel disease (IBD) has entered an exciting era, with the optimisation of existing therapies, new strategies being explored that have the potential to further improve patient outcomes, and a growing recognition of the value of a personalised approach to treatment. This symposium explored optimal approaches to using biologic therapy, and the use of therapeutic drug monitoring (TDM) and biomarkers in treatment management.

IBD shows a progressive immunopathogenesis, and a 'window of opportunity' exists whereby early intervention may alter the disease course. There is a convincing body of evidence supporting early intervention with anti-TNF-α therapies to improve patient outcomes. Cost is the major barrier to initiating and continuing treatment with biologic therapy. Biosimilars have the potential to reduce costs and increase patient access to biologic therapies, enabling more patients to receive biologic

treatment earlier. The use of TDM in the treatment of IBD is increasing and offers benefits over standardised approaches to dosing, and it is likely that emerging dose optimisation tools will enable a personalised approach to treatment in the future.

Many patients experience loss of response to anti-TNF-α therapy. Biomarkers currently used to monitor treatment response include C reactive protein (CRP), faecal calprotectin, and anti-drug antibodies (ADA). Although biomarker identification is still at an early stage for IBD, several genetic, serological, and microbiome markers have also shown promise in predicting response to anti-TNF-α therapy, while other biomarkers are also under investigation for use in diagnosis, predicting response to therapy, and treatment monitoring.

Putting the Pieces Together: When Should Anti-TNF be Introduced?

Professor Stefan Schreiber

IBD shows a progressive immunopathogenesis.[1] Following an initial immune response, amplification of this inflammatory response leads to the phenotypic expression of the disease and tissue destruction. In both Crohn's disease (CD) and ulcerative colitis (UC), T helper (Th) cells contribute to the immune response, which continues to change over time.[1] In early IBD, a Th1-driven response is dominant, whereas late IBD is characterised by a mixed Th1/Th17-driven response.[1] A better understanding of the pathogenesis of IBD has enabled the development of new therapeutic strategies targeting various inflammatory disease pathways.[2]

A 'window of opportunity' has been identified in which early treatment may have the greatest benefit.[3] However, treatment is often started a long time after diagnosis and few patients with IBD are initiated on biologic therapy. An analysis of IBD treatment pathways in the USA found that only 3.9–4.1% of patients with CD and 0.5–0.8% of patients with UC initiated treatment with a biologic,[4] with the most commonly used first-line therapies being corticosteroids (42% of patients with CD) and 5-aminosalicylic acid (35% of patients with CD and 59–64% of patients with UC). Early intervention with biologics is not appropriate for all patients and it is important to balance the potential for benefit with the risk of experiencing side effects. Prognostic factors can be used to help select patients who may benefit the most from early intervention. In patients with CD, prognostic factors for disease progression include ileal disease location, perianal disease, upper gastrointestinal involvement, and extraintestinal manifestations, together with younger age, smoking, endoscopic severity, serological reactivity to certain microbial antigens, and genetic mutations (although genetic markers are not routinely used in current clinical practice).[5]

There is a growing body of evidence supporting early intervention with anti-TNF-α, with greater treatment benefits evident in patients with a shorter duration of disease.[6-8] In the PRECiSE 2 trial in CD, patients (N=425) who responded to induction therapy and received certolizumab pegol were randomised to maintenance treatment with certolizumab pegol or placebo for 26 weeks. In the cohort treated with certolizumab pegol, response and remission rates were higher in patients with a disease duration of <1 year compared with those with a disease duration of ≥5 years. In contrast, no association between efficacy and disease duration was seen in patients treated with placebo.[6] In the CHARM and ADHERE studies, patients with moderate-to-severe CD were divided into three disease duration categories (<2 years, n=93; 2 to <5 years, n=148; and ≥5 years, n=536) and treated with adalimumab or placebo. Through all time points up to Week 164, remission rates were numerically higher in patients with disease duration of <2 years, compared with those in the longer disease duration groups. Logistical regression analyses found that remission at Week 56 was significantly associated with shorter baseline disease duration (p=0.046).[7]

In patients with UC, early success of therapy may be more important than disease duration, as rapid mucosal healing is a strong prognostic factor associated with improved outcomes.[8] In the ACT-1 and ACT-2 studies in patients with moderate-to-severe UC who received infliximab or placebo, achievement of mucosal healing at

Week 8 was associated with improvements in time to colectomy, rates of symptomatic remission, and corticosteroid-free symptomatic remission. Furthermore, patients with a greater degree of mucosal healing at Week 8 were most likely to sustain mucosal healing to Week 54.[8]

The 2-year, open-label 'Top-Down versus Step-Up' trial was conducted in patients with active CD (N=133) randomised to early combined immunosuppression (infliximab plus azathioprine; 'top-down') or conventional treatment ('step-up').[9] At Weeks 26 and 52, significantly more patients in the 'top-down' versus the 'step-up' group achieved remission. Even after 8 years, a difference between the two groups was evident in key endpoints important in the natural course of the disease. Compared with conventional step-up treatment, top-down treatment resulted in a reduced proportion of patients experiencing a flare and a longer median time to flare, although there was no significant difference in remission rates.[10] In the REACT trial, patients with CD received early combined immunosuppression (ECI; adalimumab or infliximab and azathioprine, 6-mercaptopurine or methotrexate) or conventional management (according to the centre's usual practice). In the ECI group, the presence of active disease resulted in dose escalation of the anti-TNF-α therapy. At 24 months, patient-level composite rate of surgery, hospital admission, or serious disease-related complications was lower for the ECI group than the conventional management group (hazard ratio [HR]: 0.73; p=0.0003).[11]

In the past, cost has been the major barrier to initiating and continuing treatment with biologics.[12] Biosimilars have the potential to reduce costs and increase the number of patients able to access biologic therapies, making early intervention a realistic option. In the UK, cumulative cost savings from the introduction of infliximab and etanercept biosimilars were £38.8 million between 2015 and 2017.[13] In 2015, in Norway, the prices of infliximab biosimilars were lowered to 64% of the cost of the reference product, resulting in a 34% increase in infliximab use by the following year.[14] The potential 1-year budget impact of introducing biosimilar infliximab on direct drug healthcare costs was modelled in five European countries (Belgium, Germany, Italy, the Netherlands, and the UK). A scenario in which the cost of the biosimilar was 30% lower than that of the reference product would equate to an annual saving of €35.9 million in patients with CD and €15.4 million in patients with UC, allowing 3,309 and 1,392 additional patients with CD or UC, respectively, to be treated each year.[15] Despite these clear benefits, the uptake of anti-TNF-α biosimilars varies widely across Europe (0–65% of market share).[16]

Prof Schreiber concluded that early intervention with anti-TNF-α slows disease progression and improves long-term outcomes in patients with IBD. Biologic therapy is a major cost-driver in the management of IBD, but the use of biosimilars can reduce costs and expand access, enabling more patients to receive earlier treatment.

Solving the Riddle: How Do We Implement Therapeutic Drug Monitoring to Maximise Treatment Success?

Professor Walter Reinisch

There is growing evidence for the use of TDM in IBD, whereby measurements of anti-TNF-α drug levels and antibodies against the TNF inhibitor are used to tailor therapy. This offers potential benefits over a standardised approach to dosing, enabling personalisation of therapy together with the ability to monitor treatment compliance, observe changes in pharmacokinetics (PK), reduce drug toxicity, optimise outcomes, increase cost-effectiveness, and define the biosimilarity of biologics.

Considerable interpatient variability in pre-infusion drug concentration has been observed with biologics, including infliximab and adalimumab.[17,18] Drug clearance over time and drug serum levels are key PK parameters in patients with IBD.[19] Various factors can affect these parameters, which may impact on treatment outcomes with anti-TNF-α therapies.[19] These factors include body weight/BMI, the development of ADA, inflammatory burden, serum albumin levels, and the use of immunomodulators (Figure 1).[19]

However, these PK parameters do not appear to differ between patients treated with adalimumab reference product versus a biosimilar (ABP 501).

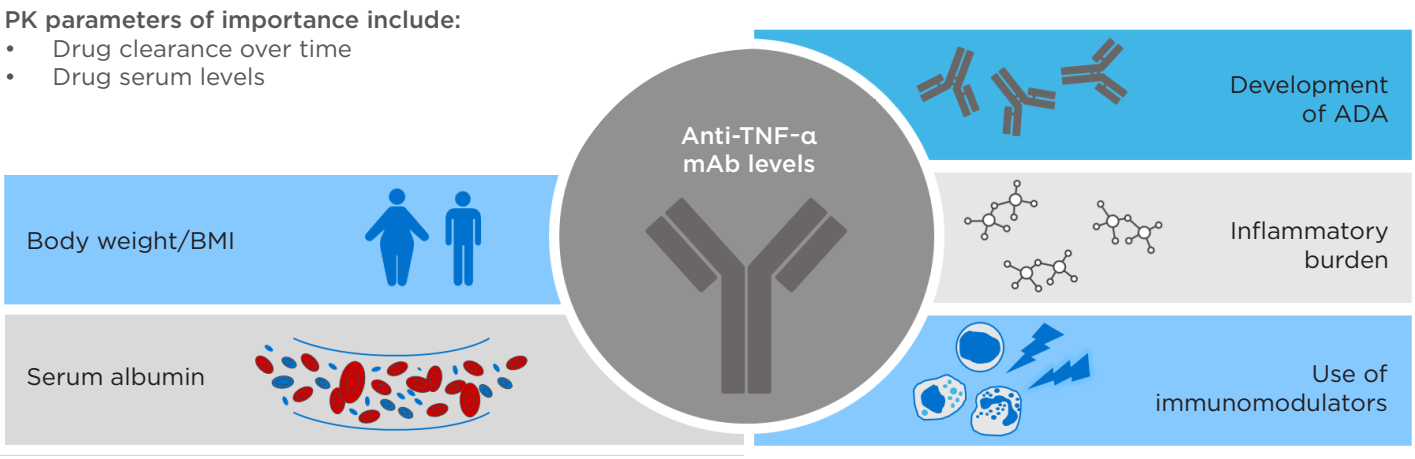

Figure 1: Factors affecting pharmacokinetic parameters in patients with inflammatory bowel disease, which may impact on treatment outcomes with anti-TNF-α therapies.

ADA: anti-drug antibody; IBD: inflammatory bowel disease; mAb: monoclonal antibody; PK: pharmacokinetic.

In a 26-week, randomised, double-blind, active comparator-controlled equivalence study of patients with moderate-to-severe active rheumatoid arthritis despite methotrexate, drug trough serum concentrations were similar in patients treated with either reference adalimumab or adalimumab biosimilar ABP 501 across all time points to Week 26.[20] The development of binding and neutralising ADA over time was also similar in patients treated with ABP 501 and those who switched from reference adalimumab to ABP 501.[21]

There is a clear exposure–response relationship for adalimumab, with higher drug serum concentrations during both induction and maintenance being associated with increased rates of remission.[22,23] In patients with UC treated with adalimumab, the probability of remission at Week 8 increased with increasing serum adalimumab trough concentrations.[22] In patients with CD, both adalimumab drug and ADA levels are predictors of clinical response.[23] A cross-sectional study showed that serum adalimumab levels above 5.85 µg/mL and ADA levels below 1.50 µg/mL–eq were associated with highest likelihood of remission.[23]

The use of TDM in the treatment of IBD is becoming increasingly common, and the current view is that proactive and reactive TDM should be considered as complementary, and not mutually exclusive, strategies.[24] Reactive TDM can potentially be used when treatment failure occurs,[25,26] to confirm that symptoms are caused by the disease, and better direct and guide patient care.[25,27] Proactive TDM may be used during induction, maintenance, or remission,[24,25,28] or in therapy de-escalation (i.e., stopping or reducing dose).[24,28] Compared with reactive TDM, a proactive approach is associated with lower rates of ADA, treatment failure, and IBD-related surgery or hospitalisations.[27] Data regarding the role of TDM during anti-TNF-α induction therapy in IBD are limited,[26] and TDM is usually applied in the maintenance setting. In a retrospective study in patents with IBD (N=264) who received infliximab maintenance therapy, proactive drug monitoring was associated with improved clinical outcomes compared with reactive monitoring, with a reduced risk of treatment failure (HR: 0.16; 95% confidence interval [CI]: 0.09–0.27; p<0.001), IBD-related surgery (HR: 0.30; 95% CI: 0.11–0.80; p=0.017), and IBD-related hospitalisation (HR: 0.16; 95% CI: 0.07–0.33; p<0.001).[27]

Prof Reinisch described a dose-optimisation tool that has recently been developed using a predictive PK algorithm based on Bayesian modelling.[29] The physician is required to enter patient factors, select a target treatment dose, and choose the dose and dosing interval via a cloud-based clinical dashboard. Using this approach, three drug serum concentration measurements can provide sufficient information for individualised dose adjustment in patients with

IBD. Assessment of long-term treatment retention showed that patients dosed in accordance with the dashboard's recommendations were more likely to remain on treatment over a follow-up period of 72 months.[29]

Looking ahead to the potential future role of TDM in IBD, it is likely that emerging dose-optimisation tools will allow a personalised approach to treatment. TDM may enable earlier identification of patients who do not have a clinical response and could provide early insights into resistance and response, which may help to profile patient populations more likely to respond to anti-TNF-α therapy and help to ensure patients receive the optimal drug dose.

Unravelling the Problem: How Can Biomarkers Be Used to Guide Clinical Management?

Doctor Gionata Fiorino

The aetiology of IBD is multifactorial, with genetic factors, lifestyle, medication, intestinal microbiota, and mucosal immunology all contributing to the heterogeneity of the disease.[30] Molecular profiling has the potential to define disease heterogeneity, identify relevant biomarkers, and ultimately stratify patient subsets who can then receive personalised healthcare in the form of disease-specific therapeutic agents. Patients in a given subset will likely still differ, but treatment with therapies targeted towards core pathways has the potential to enrich the therapeutic response.[31] IBD represents a group of patients with inherently variable disease courses. There is a changing biology from early to late stages of IBD, with the inflammatory response composed of an early Th1-driven and a late Th17-driven inflammatory response, and TNF-α produced mainly in the early stages of the disease.[1] Differences exist between patients in their inflammatory profiles due to differing disease stage (i.e., early versus late), endoscopic activity, and disease location. Understanding the expression of biomarkers across the disease course will help in selecting the most appropriate patients and targets for therapy.

Biomarker identification is still at an early stage in IBD. Biomarkers in clinical use and in development differ in their complexity and clinical utility (Figure 2).[32-38] Protein biomarkers such as CRP, faecal calprotectin, ADA, and drug trough levels show a low level of complexity, and are currently used in clinical practice to monitor inflammation and guide treatment strategy decisions.[32-34] Other more complex types of biomarker, such as mRNA, DNA, and the microbiome are currently under investigation, and could allow the identification of patients who may respond to a particular therapy.

Anti-TNF-α therapy is not effective in all patients with IBD,[39-42] and many patients experience loss of response to therapy in the long term.[43] In case series and randomised controlled trials conducted in patients with CD, the rate of loss of response at 12 months ranges from 23% to 46%.[43] Biomarkers currently used to monitor response to treatment with adalimumab include CRP, faecal calprotectin, and ADA.[26,44,45] CRP is a standard marker for the acute phase response across inflammatory diseases,[46] and can be used as a surrogate marker of active CD and an indicator of treatment response.[47] A decrease in CRP level is indicative of response to therapy, while persistently high levels of CRP are associated with diminished or loss of response.[47] Serum CRP levels may therefore be useful for assessing a patient's risk of relapse. However, CRP is a less powerful biomarker than faecal markers and patients with normal levels of CRP may have endoscopically active disease.[47] Decreasing levels of faecal calprotectin appear to correlate with clinical response and mucosal healing. Faecal calprotectin can help predict relapse and postoperative recurrence, and is useful in determining when, and in whom, a more invasive endoscopy should be performed. Faecal calprotectin has reduced value in patients with CD restricted to the small bowel, and shows an imperfect correlation with transmural inflammation.[47]

The presence of ADA is associated with negative therapeutic outcomes. There is a relationship between the presence of ADA and sub-therapeutic drug concentrations, and lower or undetectable drug concentrations are associated with treatment failure.[26] Neutralising ADA (a subset of binding ADA) can inhibit drug activity, while binding ADA can increase drug clearance.[45] Biosimilars show similar immunogenicity profiles to their reference biologics. In a 26-week randomised, double-blind

equivalence study in which patients with moderate-to-severe active rheumatoid arthritis despite methotrexate received ABP 501 or reference adalimumab (40 mg) every 2 weeks, a total of 38.3% and 38.2% of patients, respectively, tested positive for binding ADA, while 9.1% and 11.1% were positive for neutralising ADA.[20] There is limited guidance on the optimal use of ADA in treatment management and limited availability of accurate, rapid, easily administered, and inexpensive tests.[26,48]

Biomarkers may be valuable when using a treat-to-target approach, which involves predefining a treatment target associated with optimal long-term outcomes in consultation with the patient, then continuously monitoring disease activity and modifying treatment until the target is reached. All components (i.e., target, treatment, and monitoring) are tailored to the needs of the individual patient, and de-escalation of therapy may be considered when treatment goals are achieved.[49,50] CRP and faecal calprotectin are the best surrogate markers currently available for assessing endoscopic activity. The randomised, controlled CALM study demonstrated that a treatment algorithm based on these biomarkers, in conjunction with the Crohn's Disease Activity Index (CDAI) and prednisone use, resulted in better clinical and endoscopic outcomes (i.e., a greater proportion of patients achieving mucosal healing [CDAI <4] and no deep ulcers on endoscopy, deep remission, and biological remission) than symptom-driven decisions alone in patients with active, endoscopic CD, and can be used to guide treatment decisions.[51]

Several biomarkers have shown promise in predicting anti-TNF-α response. These include genetic biomarkers (e.g., polymorphisms in *FCGR3A, TLR2, TLR4, TLR9, TNFRSF1A, IFNG, IL6,* and *IL1B*), serological biomarkers (e.g., pANCA, haemoglobin, serum albumin, and TREM-1), and microbiome biomarkers (e.g., *Faecalibacterium prausnitzii* in UC).[52-54] Various other biomarkers are also under investigation for use in diagnosis, predicting response to therapy, or in treatment monitoring (Figure 3).[54-59]

In the future, it will be valuable to identify biomarkers that will help to find patients at risk of developing disease, disease progression, or complications, and to determine the right time for biologic treatment, guide treatment decisions, and select the most appropriate drugs for each individual patient.

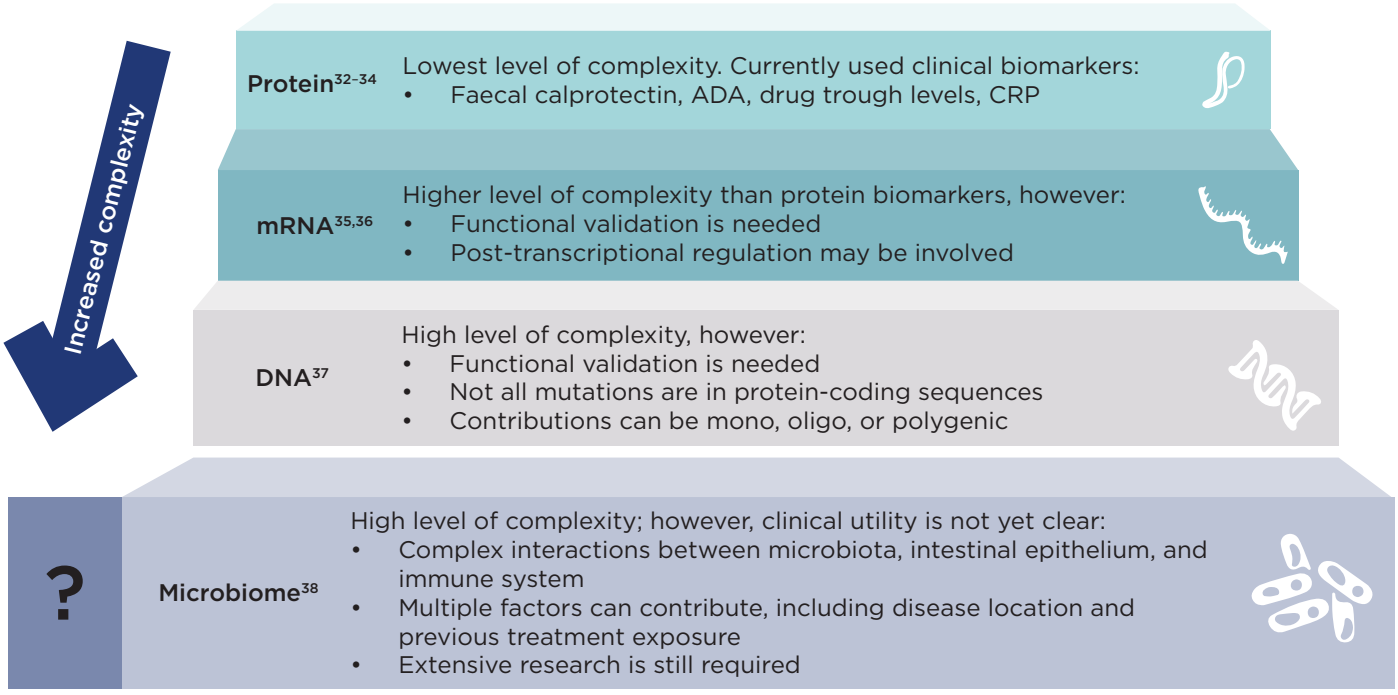

Figure 2: Different types of biomarkers have different complexity and clinical utility.

ADA: anti-drug antibody; CRP: C-reactive protein.

Diagnosis	Predictors of treatment repsonse	Monitoring of treatment
Invitae Monogenic Inflammatory Bowel Disease Panel[55] • Analyses of 47 genes that are associated with primary immunodeficiencies that lead to primarily paediatric onset IBD **Antibodies to flagellin**[56] • Possible role for anti-44-FLa2 and anti-Fla-X in distinguishing CD from UC	**Oncostatin M in mucosa**[57] • Expression of mucosal oncostatin M predicts no response to infliximab **IL-22 in blood**[58] • Higher baseline serum concentrations of IL-22 are associated with greater likelihood of response to MEDI2070 **Microbiome**[54] • Association between gut microbial taxonomic composition, and function and response to vedolizumab in CD and UC **Granzyme A and Integrin αE**[54] • Colonic expression of Granzyme A and integrin αE mRNA predicts response to etrolizumab	**Neutrophil-related markers**[59] • NGAL-MMP-9, LL-37, and CHI3L1, as well as CRP and neutrophil count, have been shown to be significantly associated with mucosal healing after adalimumab treatment in UC

Figure 3: Biomarkers under investigation in inflammatory bowel disease.

CD: Crohn's disease; CRP: C-reactive protein; IBD: inflammatory bowel disease; LL-37: cathelicidin; MMP: matrix metalloproteinase; NGAL: neutrophil gelatinase-associated lipocalin; UC: ulcerative colitis.

Concluding Remarks

A growing body of evidence supports early treatment with anti-TNF-α to slow disease progression and improve long-term outcomes in patients with IBD. However, cost is the main barrier limiting the use of biologic therapy. Biosimilars can reduce costs and facilitate increased access, enabling more patients to receive biologic treatment earlier. The use of TDM in the treatment of IBD is increasing and may enable earlier identification of patients without clinical response and help to profile patient populations more likely to respond to anti-TNF-α therapy. The future availability of dose optimisation tools also has the potential to enable a personalised approach to treatment. Biomarkers play a key role in the management of IBD. CRP, faecal calprotectin, and ADA are currently used to monitor response to anti-TNF-α therapy, and several other genetic, serological, and microbiome markers have shown promise as predictors of response. The search will continue for novel biomarkers to identify patients at risk of disease, determine the optimal time for biologic treatment, and guide treatment decisions.

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Sex Differences in Paediatric and Adult Asthma

EDITOR'S PICK

My Editor's Pick of this issue is the paper by Naeem and Silveyra on the life course of sex differences for asthma. Interestingly they observe a 'switch' from a higher incidence rate in boys to the reverse later on in adulthood when women are more likely to develop the condition. This pattern is linked to changes in hormone levels along the life course. Improved understanding of these developments will create new options for asthma treatment strategies.

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Abstract

Asthma is the most common chronic condition in Western countries. Affecting 1 in 7 children and 1 in 12 adults, asthma is responsible for >350,000 avoidable deaths every year. While most children who develop symptoms of asthma are <5 years of age, the disease is frequently misdiagnosed or not suspected in infants and toddlers. In addition, the prevalence of asthma is different in males and females throughout their lifespan. While boys are more likely to develop asthma than girls, this pattern is reversed after puberty. This indicates that sex-specific factors, such as fluctuations in hormone levels, play a role in the disease's pathogenesis. In this review, the authors discuss recent advances in diagnostic tools for asthma in both adults and children, as well as the influences of BMI, environmental exposures, socioeconomic factors, and sex hormones in the disease's pathogenesis. The review will show that both experimental and epidemiological evidence suggest that circulating sex hormone levels are important contributors to asthma symptoms in post-pubertal females, while their role in males and children has not been yet established. In addition, the mechanisms associated with these hormonal influences on airway inflammation and hyper-reactivity have not been yet elucidated. The authors conclude that different factors affect asthma rates and severity in children and adults, and that more research needs to be conducted to identify the specific contributions of sex hormones. These will allow the development of more personalised asthma treatment strategies for men and women at different stages of life.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways, characterised by obstruction of airflow, excessive mucus production, and airway hyper-reactivity. While asthma may commence at any point in life, most symptoms begin in early childhood. The pathogenesis of the disease involves a number of mechanisms in different cell types, most of which remain understudied. Inflammation is a key component of asthma and involves early and late phase responses, which are characterised by the recruitment of specific immune cells.

During puberty, asthma symptoms can ameliorate or worsen, depending on a number of factors.[1] The observed sex differences of asthma incidence in children <18 years of age in the USA (9.2% in boys versus 7.2% in girls) and in adults (6.2% in men versus 10.4% in women)[2] suggest that hormonal changes occurring during puberty may contribute to the increased incidence in adult women. This is further supported by studies indicating variation in the severity and frequency of asthma symptoms throughout the menstrual cycle.[3]

PAEDIATRIC ASTHMA: DIAGNOSIS AND MANAGEMENT

A large number of children who develop symptoms of asthma are <5 years of age.[2] Despite this, the disease is frequently misdiagnosed or not suspected in infants and toddlers.[4] Establishing the diagnosis of paediatric asthma involves a careful process of history taking, physical examination, and diagnostic studies. Coughing and wheezing are some of the most common symptoms in childhood, but shortness of breath, chest tightness, chest pressure, and chest pain are also reported. In addition, poor school performance and fatigue may also present as a result of sleep deprivation from nocturnal symptoms.

Effective asthma management in children requires preventive and proactive approaches.[4] Currently, most asthma-related visits are for urgent care. Routine follow-up visits for patients with active asthma are recommended every 1–6 months depending on the severity of asthma and management strategies, which are determined based on age group and symptoms.[4] Once the severity of asthma has been assessed, treatment is initiated and efforts are made to keep the asthma in the well-controlled category. Step-wise treatments are effective in most patients: if the symptoms aggravate, therapy is stepped up, and vice versa if symptoms improve.[5]

Clinicians use a variety of tools to diagnose asthma; for example, measurement of peak expiratory flow rate is a useful indicator of airflow obstruction, the hallmark symptom of asthma. Spirometry, which additionally measures forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), can also be used to document airflow obstruction. Spirometry can detect airflow obstruction in the presence of normal peak expiratory flow. These tests are useful tools when diagnosing asthma in adults, but the situation is different when it comes to dealing with asthma in children. In children, parents have to look out for symptoms like unresolved chronic cough, wheeze, poor sleep, irritation, and/or being restless. Thus, the first step in diagnosis is compiling a proper patient history. This is only possible if parents monitor symptoms and visit their primary care physician to get their child checked and if attention is being paid to additional factors that can accompany traditional asthma symptoms. In this regard, a number of studies have explored a variety of factors that can influence asthma diagnosis in children. These studies are summarised in Table 1.[6-13]

Recently, studies were conducted to determine if parents of asthmatic children had satisfactory levels of knowledge when it comes to their child's condition.[6] The results revealed that most parents had an unsatisfactory level of knowledge about asthma, and suggested that changes should be made in public asthma management programmes to improve parents' awareness of their child's condition, therefore allowing them to appropriately manage their child's care. It was also noted that, even after asthma was diagnosed in children, the disease was not well controlled, partly due to non-adherence. On the contrary, adherence was significantly associated with well-controlled asthma, and adherence to proper medication regimens was found to be necessary for obtaining maximum therapeutic benefits.[6]

Table 1: Clinical studies on paediatric asthma risk and management.

Reference	Study type	Sample	Method	Results	Conclusion
Roncada et al.,[6] 2018	Cross-sectional study	154 parents of children with asthma. Parents of children with asthma in remission and healthy children were also selected (control group).	The NAKQ was applied to assess the level of knowledge of parents.	Only 30.5% of parents had acceptable levels of knowledge about asthma. The mean score in NAKQ was higher in the asthma group. Surprisingly, the parents with mild and moderate asthmatic children scored higher than those of children with severe asthma (p=0.02).	Parents of asthmatic children have mediocre knowledge about asthma, which reinforces the need for changes in public asthma education and management programmes.
Frémont et al.,[7] 2018	Prospective cross-over randomised	113 babies aged 6–47 months treated with an ICS therapy. Parents who claimed that their child was not complying during therapy were asked to participate.	Children were observed during the delivery of ICS with either an animated cartoon or a black screen video. The median percentage of time of non-cooperation was compared.	Parents of 44% children reported un-cooperative children during treatment delivery. The median percentages of time of non-cooperation were significantly lower during the distraction periods (p=0.008).	Un-cooperative attitude among young asthmatic children can be amended using animated cartoons, which can increase cooperation up to 97%.
Lu et al.,[8] 2018	Case control study	37 infants with recurrent wheezing gathered from outpatient clinics, and 113 healthy infants from a longitudinal birth cohort.	Infants underwent multiple breath washout, forced expiratory flows, and body plethysmography when they were clinically stable.	LCI z-scores were higher in infants with recurrent wheeze versus healthy infants (p<0.001). 19% of recurrently wheezing infants had LCI values that were above the upper limit of normal.	Clinically stable infants with recurrent wheezing can have ventilation inhomogeneity.
Lee et al.,[9] 2018	Prospective study	1,305 elementary school children from the CHEER study.	A 4-year prospective follow-up study with 2-year intervals. Total serum IgE and percentage of blood eosinophils were measured, and allergy work-up including methacholine challenge tests and questionnaires were performed at each survey.	Early onset BHR phenotypes were associated with increased risk of newly diagnosed asthma. Late-onset BHR phenotype was associated with increased risks of allergic rhinitis symptoms at baseline and later sensitisation against inhalant allergens.	High atopic burden associates with early-onset persistent BHR phenotypes in school-aged children.
Wang et al.[10] 2018	Observational study	60 children admitted and treated in a single hospital for asthma attacks.	Comparisons among asthma patients with high/low 25-(OH)D levels and healthy children were performed based on biochemical indexes, humoral immunity, TNF-α and IL-6 levels, and pulmonary function indexes.	Serum IL-6 and TNF-α levels in the high 25-(OH)D group were lower than those in the low 25-(OH)D group at 3 days after treatment (p<0.05). 25-(OH)D had a positive correlation with pulmonary function indexes (p<0.05), while TNF-α and IL-6 were negatively associated with pulmonary function indexes (p<0.05). Serum 25-(OH)D levels in asthmatic children were negatively associated with TNF-α and IL-6 levels.	Children with asthma attacks have lower levels of 25-(OH)D, which is associated with inflammatory mediators levels as well as pulmonary function index. Thus, 25-(OH)D could be used as a test indicator for prevention and control of childhood asthma.

Table 1 continued.

Reference	Study type	Sample	Method	Results	Conclusion
Neophytou et al.,[11] 2018	Observational study	1,172 Hispanic and African-American children with asthma from the mainland USA and Puerto Rico.	Association of cotinine levels ≥0.05 ng/mL with asthma exacerbations were analysed via logistic regression analysis.	Chances of asthma exacerbation were higher in children when exposed to SHS.	The dose-responses observed show that odds of asthma exacerbation mounts with increasing exposure, even at cotinine levels associated with light SHS exposure.
Strzelak et al.,[12] 2018	Review article	ETS exposure associations with asthma and allergy in children.	Molecular mechanisms associated with inflammatory profiles aggravating inflammation, promoting infection, inducing tissue damage and promoting allergy in subjects with ETS exposure are discussed in the review.	Oxidative stress is responsible for increased mucosal inflammation and expression of inflammatory cytokines (IL-8, IL-6, and TNF-α). ETS exposure disturbs the oxidant/antioxidant balance, which results in oxidative stress.	ETS affects epithelial cells by increasing permeability, mucus overproduction, impaired mucociliary clearance, increased pro-inflammatory cytokines, enhanced recruitment of macrophages and neutrophils, and lymphocyte imbalance towards Th2.
Buelo et al.[13] 2018	Review article	Data from six databases (over 16,000 records, including 68 papers [28 cohort, 4 case-control, and 36 cross-sectional studies]).	Two reviewers independently selected studies and extracted data to perform a heterogeneity precluded meta-analysis. Weighting was undertaken by an expert panel who independently assessed each variable for degree of risk and confidence and then achieved consensus by discussion.	The risk for asthma was found greatly increased with previous asthma attacks, persistent symptoms, and poor access to care; moderately increased with suboptimal drug regimen, comorbid atopic/allergic disease, African-American ethnicity, poverty level, and vitamin D deficiency. ETS exposure, younger age, obesity, and low parental education were associated with slightly increased risk.	Clinical, demographic, and population level factors may help clinicians tailor management strategies for individual patients, as well as health service professionals and policymakers target healthcare initiatives considering population risk factors.

BHR: bronchial hyper-responsiveness; ETS: environmental tobacco smoke; ICS: inhaled corticosteroid; LCI: lung clearance index; NAKQ: Newcastle Asthma Knowledge Questionnaire; SHS: second hand smoking.

These results further support the notion that parents should be well aware of all aspects of asthma health care. In addition, the naturally non-cooperative attitude of children can affect asthma treatment efficacy. Recently, a study of cooperation in children aged 6–47 months was conducted.[7] The study found that poor cooperation among young children with asthma

was associated with poor prognosis. However, the study also revealed that cooperation could also be improved using various techniques, such as music, toys to distract children, and animated cartoons, which increased cooperation up to 97%. On the contrary, cooperation and adherence in adults was not found to be a major issue in this report.[7]

Because asthma in children presents with various nonspecific symptoms, it is difficult to assess if a child has asthma. A recent report showed that ventilation inhomogeneity is present in clinically stable infants with recurrent wheezing, and their spectrum curve indices indicated bronchodilation may be useful for the assessment of bronchial reversibility in children with asthma.[8]

In one recent study, the early-onset persistent bronchial hyper-responsiveness (BHR) phenotype in school-aged children was found to be associated with high atopic burden and increased risk of newly diagnosed asthma, whereas the late-onset BHR phenotype was related to later sensitisation and allergic rhinitis symptoms.[9] Some studies have also given their input on diagnostic criteria for asthma, which can be very helpful in children in whom spirometry cannot be performed accurately, including the use of potential biomarkers.[14] In some children with asthma attacks, circulating levels of 25-(OH) vitamin D were decreased, which was associated with the inflammatory mediators IL-6 and TNF-α, as well as altered pulmonary function.[10] This study showed that patients with allergic asthma had distinctively reduced vitamin D levels, suggesting that 25-(OH) vitamin D could be considered a biomarker for the prevention and control of childhood asthma. However, the use of vitamin D as a biomarker for asthma remains controversial and has not been established or validated in the field.[15]

PAEDIATRIC VERSUS ADULT ASTHMA

Many risk factors for the development of paediatric asthma are different to those found in adults. These differences will call for alternative preventive measures in both patient groups. For example, prevention of occupational exposures, smoking, and allergens/pollutants can help in adults, whereas avoiding certain foods and environmental exposures, such as second-hand smoking (SHS), can help in children. In both cases, asthma is mostly triggered by allergens and air pollutants. Thus, whenever a child presents to the clinic with unexplained chronic cough or wheeze, questions regarding these triggers must be asked.

A study analysing dose–response relationships of asthma and SHS in children indicated increasing odds of asthma outcomes related with increasing exposure to cotinine, even at cotinine levels associated with light SHS exposure.[11] A recent review of environmental tobacco exposure and the effect of this exposure on asthma and allergy in children indicated that oxidative stress imbalance resulting from tobacco smoke exposure can lead to mucosal inflammation and increased expression of inflammatory cytokines.[12] In addition, direct cellular effects of environmental tobacco smoke (ETS) on epithelial cells resulted in increased permeability of mucous membranes, mucus overproduction, impaired mucociliary clearance, increased pro-inflammatory cytokine and chemokine secretion, enhanced recruitment of macrophages and neutrophils, and disturbed lymphocyte balance towards Th2.[12] These mechanisms could play a major part in the pathogenesis of asthma and allergy in children; therefore, avoiding passive smoke exposure is a good preventive measure. Other factors contributing to the increased risk for asthma in children are previous asthma attacks, persistent asthma/allergy symptoms, ethnicity, and poor access to care.[16] In this regard, a recent review of six databases found a moderately increased risk for asthma as a result of suboptimal drug regimen, comorbid atopic/allergic disease, African–American ethnicity, poverty level, and vitamin D deficiency.[13] Moreover, ETS exposure, younger age, obesity, and low parental education were also found associated with slightly increased risk of asthma in children.[13]

Exposure to both indoor and outdoor air pollutants has also been associated with asthma in both children and adults.[17] For example, cooking behaviours, especially in underdeveloped nations, may contribute to the burden of particulate matter exposure in the homes of children with asthma and thus to asthma symptoms.[18] A recent study suggested that these act by altering molecular pathways,

resulting in both respiratory and cardiovascular disease.[19] In clinical studies using ozone exposures, investigators found varied responses in both children and adults, and many studies were also reviewed to see if ozone exposure contribute towards asthma pathogenesis.[20] For adult-onset asthma, long-term ozone exposure was found to be associated with varied increased risk in men versus women; however, the mechanisms of action have not been explored in detail.[21]

Genetic risk factors have also been proposed to play a role in asthma development. Accordingly, genetic contributions to asthma have been studied in various populations that identified specific gene associations and epigenetic markers associated with asthma development when the patients are <18 years of age.[22,23] One example is a study evaluating potential candidate gene–environment interactions in allergic symptoms and childhood acute lymphoid leukaemia, which found an inverse relationship between these two phenotypes.[24] The authors suggested that this inverse association between acute lymphoid leukaemia and asthma could be limited to children carrying certain genetic polymorphisms.[24] However, none of these genes have been shown to play a role in the development of asthma in adults alone. While adult lung function-related genetic variants were associated with childhood lung function, studies revealed that there was also an environmental component.[25] In this regard, some of the factors that modified the observed effects were maternal atopy, smoking during pregnancy, cigarette smoke exposure during childhood, and birth weight.[25] With regard to allergic and immune factors, a study found that children who had higher IgE levels at birth were naturally more prone to developing asthma.[26] Also, patients with monovalent IgE allergies to moulds were also found to have higher risk for asthma than patients with other allergies.[26] Their asthma was often found to be more intense and less controlled compared with that of patients with other types of allergies.

Regarding maternal factors, studies evaluating the mother's immune status during pregnancy,[27] maternal exposures,[28] perinatal exposures,[29] and the microbiome[30] suggest that the process of asthma development begins *in utero* and is independent of allergy. Accordingly, developmental exposure to endocrine disruptors, such as bisphenol A, can also alter immune function and contribute to the development of allergy and asthma, together with other diseases, such as Type 2 diabetes mellitus and cancer.[31] Thus, preventing exposure to bisphenol A may prove fruitful in avoiding the development of asthma in children, but its role in the adult population has not been studied in detail.

While many patients experience changes in asthma throughout life, asthma does not end at a certain age bracket, resulting in long term consequences in different age groups. Children with frequent asthma attacks and allergies, especially those who become adult smokers, are the most vulnerable group to show a decline in lung function when compared to normal non-asthmatic patients. Recently, the association between asthma and other chronic obstructive diseases in the adult and paediatric population was studied in 12,594 adults.[32] When chronic obstructive pulmonary disease and lung function were studied in a cohort of 53-year-old patients and correlated with childhood lung function, asthma, and smoking, it was evident that active asthma in adults is a dominant mediator in associations between childhood asthma-related risk profiles and middle-age lung function/chronic obstructive pulmonary disease.[33] Thus, appropriate interventions at a younger age may prevent patients from developing more severe obstructive respiratory disease in adulthood.

SEX DIFFERENCES IN CHILDHOOD AND ADULT ASTHMA

From the above discussion, it is obvious that differences exist in the incidence, risk factors, preventative measures, pathogenesis, presentation, prognosis, and treatment of asthma among children and adult populations. It is clear that a significant amount of work has been done to uncover the root cause of these differences to tailor preventive and treatment options for children and adults; however, little has been done to explain the differences observed in asthma incidence and presentation between male and female individuals. In the next section, the authors summarise the available literature describing this phenomenon and the potential mechanisms associated with it. The causes for the

observed sex differences in both the paediatric and adult groups remain unknown.

Data on asthma prevalence from the U.S. Centers for Disease Control and Prevention (CDC) indicated that in 2016 about 11.2% of the USA 'young teen' population (aged 12–14 years) had asthma.[2] A recent population study in children and adolescents reported that the prevalence of asthma ('asthma ever' and 'wheezing in the past 12 months') in middle school children (aged 13–14 years) was significantly higher than that in elementary school children (aged 6–7 years).[34] This study also found that the severity of asthma in girls was higher than that in boys aged 13–14 years.[34] One factor affecting these statistics is obesity and BMI; however, studies on this topic remain controversial. A study linking BMI and asthma prevalence in children aged 7–14 years showed that higher BMI increases the risk for asthma in females, whereas the inverse was true for males.[35] A similar study in adults showed that obese women had significantly higher rates of asthma than obese men and that these rates were affected by smoking status.[36] When lung function parameters were compared in children and teens with varied BMI, a lower mean FEV_1/FVC was observed among students with asthma and high BMI, which was more pronounced in boys than in girls, indicating that BMI increases the risk for asthma in boys at a younger age.[37] Together, these studies highlight the importance of BMI in paediatric and adult populations when assessing asthma, and the need for additional research in these areas to address discrepancies among studies. These data also underscore the potential role of obesity and smoking as modifiable asthma risk factors that most strongly affect adolescent women.

With regard to asthma control and quality of life, a study conducted in a Chinese cohort showed that only a minority of asthmatic adolescents reported well-controlled asthma and that poor asthma control and female sex were risk factors for low health-related quality of life.[38] In addition, this study reported different rates for asthma attacks and hospitalisations in elderly, middle aged, and young groups, but in all cases women were more prevalent and significantly more likely to have a positive allergen test than men.[38] A study focussed on the unequal prevalence of asthma in men and women showed that both the male predominance prevalence before puberty and the 'sex-shift' towards females after puberty onset were stronger in multimorbid patients who had asthma and allergic rhinitis concurrently.[39] Other studies of asthma comorbidities found adverse associations between cardiovascular disease vulnerability and the timing of asthma onset in adolescent boys.[40]

ROLE OF SEX HORMONES IN ASTHMA

Studies in animal models have suggested the involvement of sex hormones in mechanisms of lung inflammation and asthma.[41] In particular, recent studies using tamoxifen in equine asthma models have highlighted the contributions of the oestrogen receptor in lung immune cells and airway smooth muscle cell proliferation.[42] Studies in mouse models of asthma have also shown that male and female hormones can affect mechanisms of inflammation involving macrophage polarisation.[43,44] One study found that female lungs harbour greater numbers of type 2 innate lymphoid cells and more specifically a major subset of these cells lacking a killer-cell lectin like receptor G1, a population largely absent in male lungs and able to produce type 2 cytokines after sexual maturity.[45] Experiments in gonadectomised mice with hormone replacement and mice bearing either global or lymphocyte-restricted oestrogen receptor α deficiency showed that androgens rather than oestrogens regulate the number of killer-cell lectin-like receptor G1 type 2 innate lymphoid cells subset and their functional capacity.[45] Other studies have shown that Th2-mediated airway inflammation is increased by oestrogen and decreased by testosterone, and that females have increased IL-17A-mediated airway inflammation and increased dendritic cell and alveolar macrophage numbers and function when compared to males.[46] Finally, studies in human cells obtained from asthmatic patients have found the effects of oestrogen to be mediated by oestrogen receptor β activation in airway smooth muscle proliferation and signalling pathways, which may point to a novel potential target for blunting airway remodeling.[47] Together, these studies indicate that changes in oestrogen and androgen circulating levels might be the reason why asthma is more prevalent in females after reaching puberty. Overall, ovarian hormones

have been found to increase, and testosterone to decrease, airway inflammation in asthma, but the mechanisms remain unclear.

When the effects of circulating sex hormone levels were studied in asthmatic children aged 6–18 years, both beneficial effects of androgens on lung function and symptom control, and weak deleterious effects of oestradiol on lung function, were observed.[48] Starting at puberty, asthma symptoms increase in girls compared to boys, and fluctuations in hormones during menstruation are associated with changes in asthma symptoms.[49] After puberty, sex hormones have also been found to regulate asthma symptoms during menstruation, pregnancy, and menopause,[49] but studies on this topic are inconsistent and the mechanisms involved are not as clear. With regard to sensitisation and allergic responses, a recent review on immunological processes associated with IgE sensitisation concluded that female sex hormones are more likely to enhance immunological responses and exaggerated disease, whereas male hormones tend to dampen the same responses, thus increasing the risk of allergic diseases in adult females. However, these studies are controversial because female patients have also been shown to have lower IgE levels and sensitisation rates than males.[50] The observed discrepancies among studies, particularly between the results of epidemiological and experimental studies, strongly suggest that more research needs to be done to reveal the mechanisms by which sex hormones contribute to allergy and asthma in children, adolescents, and adults.

CONCLUSION

It is clear from the studies presented above that differences exist in risk factors, incidence, and progression of asthma in the paediatric and adult population. In children, challenges remain in diagnostic strategies, particularly those associated with parental education to detect symptoms. In adults, environmental exposures, socioeconomic factors, and disease comorbidities have presented challenges establishing associations that can help identify asthma risks factors. In addition, the observed sex differences in asthma incidence and severity throughout life, with asthma affecting more boys than girls before puberty, and more women than men in the reproductive years, suggest that sex hormones play important roles in the development and progression of asthma. To study the specific effects of hormones in the paediatric and adult populations, studies in children and adolescents have been conducted to address the switch in the asthma prevalence ratio but have presented with conflicting results and identified comorbidities, such as obesity and allergy, as well as environmental and genetic factors. In adults, experimental evidence from human cells and animal studies have revealed pro-inflammatory effects of oestrogen and anti-inflammatory actions of androgens. Additional findings in women presenting with variable asthma symptoms throughout the menstrual cycle have supported the hypothesis that circulating oestrogen levels influence asthma in women. Despite this evidence, the specific mechanisms of action for sex hormones to promote or prevent asthma remain unclear. More research aimed at understanding the contributions of male and female hormones, and their receptors and signalling pathways, in both lung immune and structural cells may help to identify therapeutic targets that can be used to treat asthma in male and female patients at different moments of their reproductive life.

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Epigenetics, Assisted Reproduction, and Intracytoplasmic Sperm Injection: A Review of the Current Data

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Abstract

Since the birth of the first *in vitro* fertilisation baby in 1978, >5 million babies have been born worldwide using assisted reproductive technologies (ART). ART were initially considered safe, but, in recent years, concerns regarding the association between these procedures and the increasing incidence of imprinting diseases have developed. There are numerous steps involved in ART and there are many variables that must be considered; even parental infertility may play an important role in offspring epigenetic modifications. This review presents available data from the literature regarding the incidence of these epigenetic modifications after ART, with a primary focus on oocyte insemination methodology. The authors conclude that ART, especially intracytoplasmic sperm injection, may induce epigenetic changes that can be transmitted to the offspring, but additional data are necessary to evaluate the factors involved and to determine the safety of each ART step.

INTRODUCTION

Since the birth of Louise Brown, the first *in vitro* fertilisation (IVF) baby in 1978, >5 million babies have been born using assisted reproduction technology (ART).[1] Initially, ART procedures were considered safe, but, recently, reports have shown an increased prevalence of epigenetic anomalies after assisted reproduction.

In the authors' opinion, ovarian stimulation, oxygen tension, *in vitro* maturation of oocytes, the type of culture media, the way the oocytes are inseminated (IVF or intracytoplasmic injection), the duration of embryo culture (Day 3 versus Day 5 transfer), and the transfer of fresh or thawed embryos are all factors with epigenetic potential.

There are concerns that children born using ART may have increased frequencies of diseases known to have an epigenetic aetiology; however, the effect of ART on the epigenome is unclear. The data available regarding the epigenetic effects on the offspring following ART are heterogeneous,[2] potentially due to the wide range of genes studied and to differences in the function of imprinting genes.

This article reviews important data from the literature that highlight the epigenetic changes that can occur during ART procedures. Searching the literature, the authors found studies linking ART treatment and procedures to a number of adverse obstetric outcomes, imprinting disorders, birth defects, and abnormal birth weight. Furthermore, the present review focusses on the insemination procedure, specifically the epigenetic effect of intracytoplasmic sperm injection (ICSI) upon an embryo's normal development. According to a report, ICSI was the procedure that was most likely to be associated with imprinting errors[3] due to inappropriate methylation of maternal alleles.

METHODS

Articles were identified using multiple formal search methods, which included the searching of key journals and electronic searching of main databases, including the use of free-text, index terms, and authors. Electronic searches of Web of Knowledge, Web of Science, Google Scholar, PubMed, and other databases were conducted. Free-text searches included single and plural keywords, which initially yielded a great number of articles, many unrelated to the review intended, and the articles of interest were highlighted by the authors.

DNA METHYLATION

One of the best-known epigenetic modifications is represented by DNA methylation. Methylation can be defined as the addition of a methyl (CH_3) group, modifying gene function and affecting protein expression. The most widely characterised methylation is the covalent addition of a methyl group to the C-5 position of the cytosine base. Cytosine-phosphate-guanine (CpG) islands are genomic regions containing a high frequency of CG dinucleotides. CpG islands form approximately 70% of promoters in the human genome. DNA methylation generally occurs on the cytosine residues of CpG dinucleotides through the action of several DNA methyltransferase (DNMT).

However, the DNMT process is extremely important for normal embryo development because it plays an essential role in a number of key processes, such as transcriptional repression, suppression of element transposition, imprinting genes, and X chromosome inactivation. On the other hand, methylation defects in humans are involved in various genetic diseases, including Rett syndrome or X-linked mental retardation.[4,5]

Recently, a study by Choux et al.[6] attempted to establish whether reproductive procedures could alter DNA methylation and the transcription of transposable elements and imprinted genes in the placenta and cord blood. The study included 51 IVF/ICSI singleton pregnancies, as well as 58 spontaneously conceived children, and the work focussed on umbilical cord blood and the placenta from which DNA methylation and transcription of three imprinted loci (*H19/IGF2*, *KCNQ1OT1*, and *SNURF* differentially methylated regions [DMR]) and four transposon families (LINE-1, ERVFRD, AluYa5, and ERVW) were assessed by pyrosequencing and quantitative reverse transcription-PCR.[6] The results showed significantly lower methylation levels in the IVF/ICSI group placentas compared with control groups placentas in four of the seven studied markers: *H19/IGF2*, *KCNQ1OT1*, *LINE-1H*, and *ERVFRD-1*. However, there was no difference in the cord blood results.[6]

Another key epigenetic marker that controls gene expression is covalent modification of histone proteins, and noncoding RNA. A histone modification is a covalent post-translational modification (PTM) to histone proteins, which includes methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation. These modifications can induce either gene activation or suppression depending on the nature of the modification and the specific amino acid modified. A non-coding RNA (ncRNA) is a functional RNA molecule that is transcribed from DNA but not translated into proteins. The epigenetic mechanisms are subject to environmental and developmental influences.[2]

IMPRINTING DISORDERS

Imprinting is a chemical process involving the modification of nucleotides. During gametogenesis, the process of genomic imprinting is established. The nucleus of the

zygote has an imprint memory. This memory is retained by the embryo during prenatal and postnatal life. During preimplantation development, imprinting is highly regulated. Information inherited from the previous generation must be erased in primordial germ cells to add new epigenetic information according to whether the primordial germ cell is destined to become an oocyte or a sperm cell.[7] Approximately 40 genes are known to be imprinted in humans and imprint abnormality is understood to result in 10 syndromes.

One of the greatest concerns regarding all ART treatments and procedures is an apparently higher percentage of imprinting disorders, such as Beckwith–Wiedemann syndrome (BWS: ONIM 130650) and Angelman syndrome (AS: ONIM 105830), seen in ART babies compared with the general population. Several studies were conducted to evaluate the correlation between the most common imprinting disorders and offspring born after an infertility treatment.[8-10] Besides BWS and AS, other imprinting disorders, including Prader–Willi syndrome (PWS: OMIM 176270) and Silver–Russell syndrome (SRS: OMIM 180860), showed either poor or non-existent association with ART.[11]

BWS is a congenital disorder that involves overgrowth and neoplasia.[12] It has recently gained considerable interest because the molecular cause in most cases is epigenetic, rather than genetic. BWS has been shown to involve loss of imprinting of a group of imprinted genes on 11p15. Approximately 15% of patients with BWS have aberrant methylation and imprinting of *H19* and almost half of patients with BWS have aberrant methylation and imprinting of *LIT1.*[12] The first evidence that suggested that ART is associated with BWS was published by DeBaun et al.[12] The study presented seven cases of children born after ART, all of whom were diagnosed with BWS. ICSI was performed in five cases, and in the other two cases conventional IVF was used. Molecular studies of six of the children indicate that five of the six had specific epigenetic alterations associated with BWS, four at *LIT1* and one at both *LIT1* and *H19.*[12]

In the following years, other authors, among them Rossignol et al.,[13] supported the same conclusion. On the other hand, a Danish National IVF Cohort Study followed 442,349 singleton non-IVF children and 6,052 IVF children[14] and concluded that no imprinting disorder was found in the IVF cohort. An Irish study published in 2007 supported that there is a small risk of imprinting diseases among ART conceived children.[15]

A recent study published in 2016 by Tenorio et al.[16] followed and assessed 187 children with BWS, conceived naturally or following ART. The researchers concluded that there was a link between molecular aetiology of the disease and the type of conception, and that the odds ratio for BWS in children conceived by ART is 7-fold higher than babies conceived naturally. In addition, the hypomethylation of KCNQ1OT1:TSS-DMR was present in the ART group, while it was observed in approximately 50% of cases in the spontaneous conception group.

AS affects approximately 1 in 16,000 children, and it is characterised by severe intellectual disability, speech impairment, ataxia, a happy demeanour, seizures, and microcephaly.[17] Approximately 3% of patients with AS have an imprinting defect, evidenced by a paternal-only pattern of methylation. AS was first related to ART in general, and to ICSI in particular, in 2002 in a study conducted by Cox et al.[3] reporting two cases of ICSI treatment followed by AS diagnosis. ART has been implicated in AS by reports of five ART-conceived patients with epimutation-AS.[3,8,18] Of these, four were conceived using ICSI[3] and one using ovarian hyperstimulation alone.[8] However, the literature data are inconclusive and need further investigation. A Danish survey from 2005 concluded that in 25,000 offspring born after IVF no cases of AS were found.[14] In the same year, another German study suggested, for the first time, a possible link between subfertility, by itself, and the increased number of AS diagnosis among children born after infertility treatment.[8] In their study, Ludwig et al.[8] found no difference regarding the relative risk of an imprinting disorder between the infertile couples who were treated by ICSI or hormone therapy and the subfertile couples who did not undergo a fertility treatment. Furthermore, another study by Doornbos et al.[19] established that the major risk factors for infertility treatment related to AS were long-term infertility and ovulation induction.[19]

PWS affects approximately 1 in 17,500 children and is characterised by neonatal hypotonia, childhood onset obesity, cognitive impairment, distinctive behavioural characteristics, hypogonadism, and a characteristic facial appearance.[20] There is no single gene responsible for PWS, but most aspects of the PWS phenotype result from the absence of paternal expression of a cluster of non-coding RNA known as 'HBII-85'.[21]

SRS is a disorder of decreased growth that is estimated to affect 1 in 100,000 children. SRS is characterised by intrauterine and post-natal growth retardation plus variable additional features, including fifth finger clinodactyly, limb length asymmetry, a typical facial phenotype, and variable learning disabilities.[22] SRS differs from other imprinting syndromes in that three distinct imprinted loci on two different chromosomes have so far been implicated.[22,23] There is currently little evidence linking SRS with ART. To date, there have been five patients reported with SRS and who were conceived using IVF or ICSI[23] and molecular data are available for only two. One ICSI-conceived girl with an SRS-like phenotype was found to have hypomethylation at the paternal allele.[23]

Three publications have indicated the existence of a novel imprinting syndrome resulting from maternal hypomethylation at multiple loci.[13,23,24] Results indicate that the maternal hypomethylation syndrome can be associated with, but is not limited to, ART conceptions.[7]

In a more recent meta-analysis, published in 2018, Cortessis et al.[25] reviewed 23 studies from the literature concentrating on the correlation between ART and imprinting diseases incidence and concluded that there was a positive association among them.

In conclusion, evidence of imprinting syndromes resulting from epimutations in ART-assisted pregnancies is so far confined to three syndromes: BWS, AS, and the maternal hypomethylation syndrome. It is notable that for all three syndromes the observed epigenetic defect is hypomethylation on the maternal allele.

IN VITRO FERTILISATION VERSUS INTRACYTOPLASMIC SPERM INJECTION

ICSI is a procedure widely used for achieving fertilisation of oocytes. The procedure was first described by Palermo et al.[26] in 1992 and represents a major advance in infertility treatment for couples for whom classical IVF is not an option due to low sperm count. The technique involves the injection of a single sperm cell into the oocyte.[26] There have been many studies to date concerning the safety of this procedure. The present paper reviews the data available in the literature concerning the link between ICSI and epigenetic modifications.

Over the years, a lot of theories regarding IVF or ICSI-born children have been suggested but the debate continues with more studies performed and interest in epigenetic activity increasing.

In a retrospective cohort study of children born between 2002 and 2008, Whitelaw et al.[27] measured the DNA methylation in paternally expressed gene 3 (*PEG3*), insulin-like growth factor II (*IGF2*), *SNRPN*, long interspersed nuclear element I (*LINE I*), and the insulin gene (*INS*) in buccal cell DNA obtained from children born following IVF (n=49) and ICSI (n=20) procedures and then compared them to the spontaneously conceived children (n=86). The characteristics of the spontaneously conceived group were matched as closely as possible to the ART group and there were no significant differences in any of the subject characteristics. The results showed no significant differences between the children born using ART and the control group for three of the four genes studied and for the repeat element. The only significant difference was related to the *SNRPN* methylation, which was significantly higher in the ICSI group compared to the spontaneous conceived group. The difference remained important in a comparison between ICSI and standard IVF, and between ICSI and combined IVF and control groups. Additionally, higher levels of *SNRPN* methylation was associated with a longer infertility duration.

Another study, conducted by Rancourt et al,.[28] aimed to establish if there is a connection between the method of conception and an

increased risk of rare childhood disorders. The study collected data and biospecimens (placenta and umbilical cord samples) from three groups of women, 61 of whom conceived spontaneously, 59 of whom conceived by IVF, and 27 of whom conceived by ovulation induction. The population studied was restricted to non-Hispanic, white individuals, because of the evidence that methylation may vary with race,[5] and to singleton births resulting from one implanted placenta. To analyse the role of the conception method in the epigenetic activity, six DMR were examined by bisulfite pyrosequencing in both the cord blood (embryonic) and the placenta (non-embryonic). The authors observed that the methylation levels of *GRB10*, *MEST*, *H19*, *SNRPN*, and *KCNQ1,* as well as *IGF2*DMR0, were not disrupted by the fertility treatments. However, the methylation levels for *H19* were marginally lower in placentas from children conceived by fertility treatment. Although the study did not consider the different ART procedures in detail, the conclusions are reassuring for infertile couples.[28]

A study reported by Tierling et al.[29] concluded that there was no significant difference between the epigenetic effects of IVF and ICSI, but the researchers followed a more heterogeneous population, including twin pregnancies.

Sutcliffe et al.[9] suggested that there is a strong link between different types of ART (IVF and ICSI) and imprinting syndromes. However, due to the fact that this study was underpowered and suffered from methodological difficulties, the research lacked reliable data on this subject. The incidence of imprinting disorders overall is small (<1:12,000 births).[30]

Kobayashi et al.[31] showed that 14% of infertile men had an abnormal methylation imprints in their sperm. The outcome of ART with sperm shown to have an abnormal DNA methylation pattern was generally poor. Their data suggested that sperm from infertile patients, especially those with oligozoospermia, may carry a higher risk of transmitting incorrect primary imprints to their offspring, highlighting the need for more research into ART.

Additionally, a study by Marques et al.[32] showed that 30% of men with severe oligozoospermia had an altered *H19* methylation profile. The data suggested an association between abnormal genomic imprinting and hypospermatogenesis, and that spermatozoa from oligozoospermic patients carry an increased risk of transmitting imprinting errors. Ørstavik et al.[18] concluded that ICSI can lead to an increased risk of imprinting defects. Cox et al.[3] concluded that there are some indications that ICSI might interfere with the establishment of the maternal imprint in the oocyte or pre-embryo and increase the risk of imprinting defects. Estill et al.[33] concluded that ICSI culture conditions and parental infertility itself have a lasting impact on a child's epigenome.

There are studies published asserting that the epigenome of ART children remains essentially unchanged.[34-36] Santos et al.[37] concluded, following research on 76 ICSI embryos, that this insemination procedure does not lead to an increased incidence of epigenetic errors.[37] The study showed that DNA methylation pattern was consistent with the normal one, up to the blastocyst stage. Santos et al.[37] found no evidence that blastocysts obtained from injected oocytes were more severely affected than those obtained from conventional insemination. Ghosh et al.[38] compared methylation of CCGG sites in the placentas between ICSI and IVF and no significant differences were observed. The study published by El Hajj et al.[39] on DNA methylation signatures in the cord blood of ICSI children raised concerns that ART-induced epigenetic changes may be transmitted to the offspring, conferring a higher risk of imprinting and other disorders. To study the possible impact of ICSI on the epigenome of the exposed offspring and to identify susceptible loci, El Hajj et al.[39] compared the cord blood methylomes of healthy ICSI newborns versus naturally conceived newborns, using 450,000 methylation arrays. The observed methylation patterns in both the ICSI and the control group were within the normal range of methylation variation.

CONCLUSIONS

The continuous development of ART procedures and treatment has led to more and more ART-conceived children; however, with this rise has come growing concern about the safety of these techniques. When ICSI was first introduced it was intended to help oligozoospermic males to

conceive, but nowadays this technique is used worldwide in many cases that do not involve male infertility. Therefore, it has become very important to establish the possible side effects on the embryos and future children conceived by IVF-ICSI. Many scientists have concentrated on retrospective and prospective follow-up studies of babies born after an infertility treatment to determine the possible alterations of the embryo genome and the risk of rare malformation or genetic diseases.

Molecular analysis permits the identification of various gene defects due to epigenetic abnormalities, that may lead to imprinting disorders, such as BWS, AS, and PWS, or to defects of methylation-related syndromes, like Rett syndrome. Some authors have associated these disorders with infertility procedures and treatment, reporting a significantly increased prevalence in ART-conceived offspring. Meanwhile, other studies showed no difference between the general population and infertile couples or linked the genetic disorders to long-term infertility and ovulation induction, rather than to ART treatment.

Based on the available literature data, it is difficult to conclude that there is a strong correlation between ICSI and these epigenetic syndromes. Furthermore, all of the parameters involved, including infertility duration, ovulation induction, oocyte retrieval, fertilisation, and various lab variables, need to be taken into account.

At the same time, epigenetic abnormalities can be found in a large and heterogeneous variety of genes leading to the need for more exact studies. Additionally, there is a lack of long-term follow-up studies due to the fact that the phenotypes associated with epigenetic disorders are sometimes difficult to establish early in life or may be very subtle, for example predisposing neoplasia.

In conclusion, the available data suggest an association between ART overall and the incidence of three imprinting disorders: BWS, AS, and maternal hypomethylation syndrome. ICSI may be a technique that can lead to a higher incidence of imprinting disorders, but additional data is necessary to evaluate the factors involved and to determine the safety of every single ART step.

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Immunotherapy in Small Cell Lung Cancer

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Abstract

Small-cell lung cancer (SCLC) is extremely sensitive to standard treatments, including conventional cytotoxic chemotherapies and radiotherapy, and has poor prognosis and short survival. Standard therapies have reached a plateau of effectiveness and new therapeutic strategies are needed to improve SCLC patient outcomes going forward. Immunotherapy has revolutionised the treatment of solid malignancies, offering a novel way to harness the host immune system to target malignant cells in patients whose disease may no longer respond to cytotoxic therapy. This review describes the available data for the checkpoint inhibitors, such as anti-cytotoxic T-lymphocyte antigen-4 protein (CTLA-4), anti-programmed cell death-1 protein receptor (PD-1), and ligands (PD-L1 and PD-L2) alone or in combination with first-line chemotherapy or in relapsed SCLC. Several trials investigating immunotherapy in SCLC patients are ongoing and the results are awaited soon. Moreover, further immune checkpoint inhibitors directed against other targets, such as the killer-cell immunoglobulin-like receptor and lymphocyte-activation gene-3, are in clinical development.

Overall, the high expectations from the oncology community are that the drugs under development will offer new and improved treatment options for SCLC patients.

INTRODUCTION

In approximately 15% of cases, new lung cancer patients are diagnosed as having a small-cell lung cancer (SCLC), and in about 70% of these cases the diagnosis is performed at the extensive stage of disease.[1] SCLC is closely associated with the intensity and duration of tobacco smoking, and due to the changing smoking patterns of the last decades, the peak of its incidence has been slowly decreasing since the 1980s.[1,2] This is due to the change in tobacco habits with the use of efficient filter tips, highly porous cigarette paper, and changes in the composition of the tobacco blend.[2]

The two stage system, proposed by the Veterans Affairs Administration Lung Study Group (VALG), classified SCLC into limited disease (LD), confined to one hemi-thorax, with or without regional lymph node involvement, which could be encompassed in one radiation field, and extended disease (ED), which is not encompassable in a tolerated radiation field and includes malignant pleural effusion and distant metastasis.[3] The VALG staging system is helpful in decision-making but there is a significant difference in prognosis within the LD and ED groups. Thus, the International Association for the Study of Lung Cancer (IASLC) recommended a new staging system based on the American Joint Committee on Cancer (AJCC) TNM for the precise staging of SCLC.[4]

The multimodality approach is the standard-of-care for LD-SCLC patients with concurrent chemotherapy, platinum plus etoposide regimen, and thoracic radiotherapy. This approach should be offered to all patients with a good performance status and adequate organ function.[5] Sequential chemoradiotherapy should be considered for LD-SCLC patients who are not fit for concurrent chemoradiotherapy. Prophylactic cranial irradiation should be considered for LD-SCLC patients who achieve disease control after induction chemoradiotherapy.[6] The median overall survival (OS) of LD-SCLC patients is about 15–20 months with 2 and 5-year survival rates of 20–40% and 10–20% respectively.[7]

The standard first-line treatment for ED-SCLC patients is 4–6 cycles of mainly platinum plus etoposide regimen, followed by active surveillance. Prophylactic cranial irradiation could also be considered for ED-SCLC patients who achieve disease control after induction chemotherapy.[6] Median OS for ED-SCLC is about 8–13 months, with 2 and 5-year survival rates of approximately 5% and 1–2%, respectively.[7]

Overall, despite the fact that SCLC is extremely sensitive to standard therapies, including conventional cytotoxic chemotherapies and radiotherapy, it has poor prognosis and short OS. In fact, SCLC is a very aggressive disease, characterised by a rapid doubling time, high growth fraction, paraneoplastic syndromes, and the early development of widespread metastases, with most of these relapsing within 1 year with an OS <6 months.[1] Patients treated with a first-line platinum-based regimen at relapse can be empirically divided into 'refractory', i.e., those who progress during first-line treatment; 'resistant', i.e., those who show initial response to treatment but progress within 3 months of completing treatment; and 'sensitive', i.e., patients who have a relapse-free interval of at least 3 months from completion of treatment.[8] The objective response rate (ORR) of second-line therapy ranges from 10–25% for resistant and sensitive disease, respectively, with topotecan being the only globally approved agent in this setting.[9]

Considering this scenario and to improve outcomes for SCLC patients, new approaches, in particular the potential role of immunotherapy, are under investigation with interesting preliminary results already available. Research in the field of oncology has seen an increase in our ability to harness the host immune system to target malignant cells in a more sophisticated and effective way. This review will focus on the existing data for immunotherapy in SCLC, including immune checkpoint inhibition and exploring correlated emerging biomarkers.

IMMUNOTHERAPY: GENERAL CONSIDERATIONS

Immunotherapy in the management of cancers aims to stimulate immune responses to inhibit the tumour from escaping immune surveillance. To date, two main checkpoints have already been well characterised, including the cytotoxic T-lymphocyte antigen-4 protein (CTLA-4) and programmed cell death-1 protein receptor (PD-1) and ligand (PD-L1 and PD-L2) pathways.

CTLA-4 is a critical negative molecule in the checkpoint pathway that plays an important role in regulating the early activation and proliferation of the T-cell activity peripherally in lymph tissue.[10] Two antibodies targeting the CTLA-4 receptor are being investigated in patients with SCLC: ipilimumab and tremelimumab.[11]

PD-1, also expressed by activated T-cells, engages with PD-L1 and PD-L2 ligands, defining a checkpoint pathway involved in suppressing autoimmunity during T-cell activation, allowing for the immune tolerance of PD-L1 expressed cells at the site of the tumour.[10] Among the anti-PD-1 inhibitors, two monoclonal antibodies are in advanced stages of clinical development for SCLC: nivolumab and pembrolizumab. Among anti-PD-L1 inhibitors, another two monoclonal antibodies are in late clinical trials investigation for SCLC: atezolizumab and durvalumab.[11]

Taking this into account, SCLC is associated with a high non-synchronous mutation burden, a characteristic typically consistent with other cancers exhibiting an excellent anti-tumour response to checkpoint inhibition. This provides a strong rationale for the development of immunotherapy studies in SCLC.[12]

Although many cancer patients respond well to immune checkpoint blockade and show an improved OS, there are still patients who do not benefit from immunotherapy. Hence, patient

selection is a goal to pursue steadily to optimise immunotherapy outcomes.

PD-L1 expression as a biomarker to select patients who could greatly benefit from immunotherapy has been investigated in several solid tumour types, especially in non-small cell lung cancer, even if some patients who are PD-L1-negative based on immunohistochemistry responded to treatment.[13] However, while pembrolizumab was approved for the treatment of advanced non-small cell lung cancer patients based on PD-L1 expression (≥50% for first-line therapy and ≥1% for second-line treatment), nivolumab and atezolizumab were licensed for second-line therapy regardless of PD-L1 expression.[14] PD-L1 expression is also influenced by a dynamic tumour microenvironment and should be considered as a surrogate of a very complex system. For this reason, the identification of further biomarkers for patient selection, such as the non-synonymous mutation burden, molecular smoking signature, mismatch-repair deficiency of tumours, tumour infiltrating lymphocytes, IFN-γ expression, and intrinsic driver mutations, is necessary to optimise checkpoint inhibition results.[15,16]

FIRST-LINE TREATMENT

A Phase II randomised trial with three arms investigated carboplatin/paclitaxel regimen plus placebo for 6 cycles; same regimen plus concurrent ipilimumab (10 mg/kg, every 3 weeks), a human IgG1 monoclonal antibody anti-CTLA-4, administered with the first 4 cycles of chemotherapy; or chemotherapy plus 'phased' ipilimumab administered with the last 4 cycles of treatment. These regimens were followed by maintenance ipilimumab or placebo every 12 weeks. A total of 130 patients with ED-SCLC were enrolled. The trial's endpoints included progression-free survival (PFS), immune-related PFS, ORR, immune-related ORR, OS, and safety. The phased schedule of ipilimumab showed better results than the concurrent one and the control arm with grade ≥3 immuno-related toxicities of 17%, 21%, and 9% for phased ipilimumab, concurrent ipilimumab, and control group, respectively.[17]

Based on this Phase II randomised trial, phased ipilimumab was investigated in the following Phase III study.[18] In this trial, 1,132 patients were assigned to a platinum/etoposide regimen for 4–6 cycles plus phased ipilimumab 10 mg/kg or placebo every 3 weeks followed by a maintenance phase of ipilimumab 10 mg/kg or placebo every 12 weeks until progression. The primary endpoint was OS, and there was no difference between the chemotherapy/ipilimumab versus the chemotherapy/placebo arms. No subgroups demonstrated greater benefit with the addition of ipilimumab versus chemotherapy alone. Grade ≥3 toxicity was higher in the chemotherapy/ipilimumab arm compared to the control group.[18]

A Phase II study evaluated phased ipilimumab in combination with a carboplatin/etoposide regimen followed by maintenance ipilimumab every 12 weeks until progression in 42 ED-SCLC patients, of whom 39 were evaluable for safety and 38 for efficacy.[19] The primary endpoint was 1-year PFS. In this single-arm study, the evaluation of autoantibody serum levels was planned and correlated with clinical outcomes. Detection of autoantibodies was performed at baseline and during follow-up if clinically indicated and comprised SRY-box 2, anti-human, purkinje cell cytoplasmic antibody type 1, voltage-gated calcium channel antibody, anti-voltage gated potassium channel antibody, anti-nuclear antibody, antineutrophil cytoplasmic antibody, thyroid peroxidase, rheumatoid factors, and anti-muscle antibodies. The 1-year PFS was 15.8%, with five deaths related to ipilimumab. Positivity of an autoimmune profile at baseline was associated with improved outcomes and severe neurological toxicity.

Overall, the lack of benefit from ipilimumab addition to chemotherapy in ED-SCLC might be partially explained by the potential mechanism of chemotherapy-induced immunosuppression, which limits T-cell proliferation, or low T-cell activation within the tumour microenvironment. The treatment benefit correlated to autoantibody analysis; however, as this has been based on a limited number of patients, it should be further investigated.

On the other hand, PD-1 inhibitors targeting tumour-infiltrating lymphocytes, in respect to the anti-tumour activity of CTL-4 inhibitors, act through nonredundant pathways.[10]

Atezolizumab, a fully humanised, engineered monoclonal antibody of IgG1 isotype anti-PD-L1, was investigated in combination with chemotherapy. The IMpower-133 study was a Phase I/III randomised, double-blind, placebo-controlled trial in which 403 ED-SCLC patients were randomised to receive carboplatin/etoposide plus atezolizumab, at the flat dose of 1,200 mg, or placebo for 4 courses recycled every 3 weeks, followed by maintenance atezolizumab or matched placebo until progression or inacceptable toxicity. Co-primary endpoints were PFS and OS. The median OS was 12.3 months in the atezolizumab group and 10.3 months in the placebo group while the median PFS was 5.2 and 4.3 months, respectively. Atezolizumab did not increase the toxicity related to chemotherapy adding immune-related adverse events which were consistent with the previously reported safety profile of the drug.[20] Table 1 summarises the main results of first-line immunotherapy trials.

Considering the results of the IMpower-133 trial, the addition of atezolizumab plus carboplatin/etoposide can be considered as a new potential standard-of-care for first-line therapy of ED-SCLC. However, the results of other ongoing trials with the same study design are awaited and may help in defining whether immunotherapy plus chemotherapy is appropriately transferable to all ED-SCLC patients in daily clinical practice.

STRATEGIC MAINTENANCE APPROACH

To date and in this setting, the results of only one trial are currently available. A Phase II study investigated pembrolizumab, administered at the flat dose of 200 mg every 3 weeks as maintenance therapy. A total of 45 unselected ED-SCLC patients who did not progress to first-line therapy were enrolled. The primary endpoint was PFS, which was 1.4 months with a 1-year PFS of 13.0%. The median OS was 9.6 months with a 1-year OS of 37.0%. The ORR was 8.9%. Tumour tissue, available for 20 patients, was assessed for PD-L1 expression both in the tumour cells and in the surrounding stroma.

Table 1: Results of the main first-line immunotherapy trials in ED-SCLC.

Author	Phase of study	Treatment	No. pts	ORR (%)	PFS (months)	OS (months)	Grade ≥3 toxicity (%)
Reck et al.,[17] 2013	IIR	PCb+PLB	45	49.0	5.2	10.5	9.0
		vs					
		PCb+C-I	43	32.0	3.0	9.1	21.0
		vs					
		PCb+P-I	42	57.0	5.2	12.5	19.0
Reck et al.,[18] 2016	III	CE+PLB	476	62.0	4.4	10.9	44.0
		vs					
		CE+P-I	478	62.0	4.6	11.0	48.0
Arriola et al.,[19] 2016	II	CbE+P-I	39	72.4	6.9	17.0	89.7
Horn et al.,[20] 2018	III	CbE+PLB	202	64.4	4.3	10.3	57.6
		vs					
		CbE+A	201	60.2	5.2	12.3	58.1

A: atezolizumab; CbE: carboplatin plus etoposide; CE: cisplatin plus etoposide; C-I: concurrent ipilimumab; ED-SCLC: extensive disease small cell lung cancer; IIR: Phase II randomised; ORR: objective response rate; OS: overall survival; PCb: paclitaxel plus carboplatin; PFS: progression-free survival; P-I: phased ipilimumab; PLB: placebo; Pts: patients.

A sample was considered adequate for PD-L1 assessment only if there were at least 50 viable tumour cells or 5 viable tumour cells with PD-L1 staining. The stromal interface was considered positive for PD-L1 if a lichenoid pattern of PD-L1 membrane-stained cells surrounding the tumour nests was identified at low power. The median PFS in the eight patients with tumours positive for expression of PD-L1 at the stromal interface was 6.5 months compared with 1.3 months in 12 patients with tumours negative. Serious adverse events included two patients with acute coronary syndrome. The only Grade 3 toxicity that occurred was hyponatraemia in four patients.[21]

The results reported by pembrolizumab in the small group of patients with tumours positive for expression of PD-L1 at the stromal interface could be used as hypotheses generating in selecting patients who could benefit from this strategic approach. However, the maintenance strategy is still being investigated by other ongoing trials.

SECOND AND LATER-LINE TREATMENT

KEYNOTE-028, a multicohort Phase Ib study, investigated pembrolizumab at the dose of 10 mg/kg, every 2 weeks, for ≥2 years or until progression or intolerable toxicity in 24 patients with PD-L1 expressing (≥1%) ED-SCLC after progression on platinum based chemotherapy, in second or third-line treatment. PD-L1 positivity was defined, with the availability of at least 50 viable neoplastic cells, by membranous PD-L1 expression (≥1%) of tumour and associated inflammatory cells or positive staining in stroma. Pembrolizumab showed an ORR of 37.5% with a median PFS and OS of 1.9 and 9.7 months, respectively. Treatment related toxicity was reported in 66.7% of cases with Grade 3–5 adverse events in 8.3% of patients.[22]

CheckMate-032 is a Phase I/II study evaluating nivolumab as a single agent or in combination with ipilimumab in pre-treated SCLC.[23-25] Patients were randomised to receive nivolumab 3 mg/kg every 2 weeks or nivolumab + ipilimumab ([1 mg/kg + 3 mg/kg] or [3 mg/kg + 1mg/kg] every 3 weeks for 4 cycles, then nivolumab 3 mg/kg every 2 weeks). The primary endpoint was the ORR which, in the intention-to-treat population of 216 patients, was higher in the combination arms versus nivolumab alone (23% [nivolumab 1/ipilimumab 3], 19% [nivolumab 3/ipilimumab 1], and 10%, respectively), and this was observed regardless of PD-L1 expression. Tumour PD-L1 expression was categorised as positive when staining of tumour-cell membranes (at any intensity) was observed at prespecified expression levels (≥1% or ≥5% of tumour cells in a section that included ≥100 evaluable tumour cells). Median PFS was 2.6 months in the nivolumab 1/ipilimumab 3 arm and 1.4 months in both the nivolumab alone and nivolumab 3/ipilimumab 1 cohorts. Median OS was 7.7 months (nivolumab 1/ipilimumab 3), 6.0 months (nivolumab 3/ipilimumab 1), and 4.4 months (nivolumab alone). A higher rate of any grade treatment related toxicity was observed in the combination arms (74–80% and 58%; Grade 3–4: 18–30% and 13%, respectively).[23] The CheckMate-032 trial also included a Phase II randomised part comparing nivolumab 3 mg/kg versus nivolumab 1 mg/kg plus ipilimumab 3 mg/kg. In the combination and nivolumab arms the ORR was 25% and 11% with a median OS of 7.9 and 4.1 months, respectively. The safety was consistent with previously reported results.[24]

The results of the subgroup of 109 SCLC patients enrolled in the CheckMate-032 study in third or later-line nivolumab monotherapy 3 mg/kg every 2 weeks, until disease progression or unacceptable toxicity were also reported. The ORR was 11.9% with a median duration of response of 17.9 months. The 6-month PFS was 17.2% with a 12-month and 18-month OS of 28.3% and 20.0%, respectively. Grade ≥3 toxicities occurred in 11.9% of patients and 3 patients (2.8%) discontinued the therapy due to treatment-related adverse events.[25]

Based on these results the U.S. Food and Drug Administration (FDA) granted accelerated approval to nivolumab, at the flat dose of 240 mg every 2 weeks, for patients with SCLC progressed after platinum-based chemotherapy and at least one other line of therapy.[26]

Potential biomarkers of interest in predicting response to checkpoint blockade in SCLC include tumour mutational burden (TMB) and PD-L1 expression. The whole exome sequencing of 211 SCLC patients from the

nonrandomised or randomised cohorts of CheckMate-032 was used to evaluate the impact of TMB on efficacy of nivolumab alone or combined with ipilimumab.[27] Patients were stratified into low TMB (0–<143 mutations), medium (143–247 mutations), and high (≥248 mutations). Within both the nivolumab alone and combined with ipilimumab treatment groups, ORR was higher in patients with high TMB (21.3% and 46.2%) than in those with low (4.8% and 22.2%) or medium (6.8% and 16.0%) TMB. The 1-year PFS was higher in the high TMB group (21.2% and 30.0% for nivolumab monotherapy and nivolumab plus ipilimumab, respectively) compared with the low (not calculable and 6.2%) or medium (3.1% and 8.0%) TMB groups. The 1-year OS was higher in the high TMB group (35.2% and 62.4% for nivolumab monotherapy and nivolumab plus ipilimumab, respectively) than in the low (22.1% and 23.4%) or medium (26.0% and 19.6%) TMB groups. This exploratory data suggested that the high TMB tertile could be considered a predictor of activity, particularly for the combination of nivolumab plus ipilimumab rather than more generally prognostic in patients with SCLC.

Preliminary results from other ongoing trials showed a low activity of immunotherapeutics despite durable clinical activity in certain patients, which needs to be well defined.[28-30] Considering all these results together, the role of further line checkpoint inhibitors becomes much less positive, but the results of the ongoing trials should clarify this.

PD-L1 expression was found in SCLC to range from 0–80%.[31-34] This high discrepancy between the several case series might be explained by several reasons, such as the different scoring methods, different antibodies, different immunoreactivity in formalin-fixed, paraffin-embedded tissues, delayed or prolonged or inadequate fixation, inadequate fixatives, or pathological interpretive/analytical factors.[34]

Results from clinical trials investigating immune checkpoint blockade in second and later-line treatment in SCLC patients are summarised in Table 2.

A press release announced that the CheckMate-331 study did not meet its primary endpoint. The CheckMate-331 was a Phase III randomised open-label study comparing nivolumab versus topotecan or amrubicin in SCLC patients who failed first-line platinum-based chemotherapy. The primary endpoint was OS and was not met, with the secondary endpoints being PFS and ORR.[35] The final results will be presented in the forthcoming months.

Table 2: Results of the main second or later-line immunotherapy trials in SCLC.

Author	Phase of study	Treatment	No. pts	ORR (%)	PFS (months)	OS (months)	Grade ≥3 toxicity (%)
Ott et al.,[22] 2017	Ib*	Pembrolizumab	24	33.3	1.9	9.7	33.3
Antonia et al.,[23] 2016	I/II	Nivolumab 3 mg/kg	98	10.0	1.4	4.4	13.0
		Nivolumab 1 mg/kg + I 1 mg/kg	3	33.0	-	-	0.0
		Nivolumab 1 mg/kg + I 3 mg/kg	61	23.0	2.6	7.7	30.0
		Nivolumab 3 mg/kg + I 1 mg/kg	54	19.0	1.4	6.0	19.0
Hellmann et al.,[24] 2017	IIR	Nivolumab 3 mg/kg	98	11.0	NR	4.1	14.0
		vs					
		Nivolumab 1 mg/kg + I 3 mg/kg	61	25.0	NR	7.9	33.0

Table 2 continued.

Author	Phase of study	Treatment	No. pts	ORR (%)	PFS (months)	OS (months)	Grade ≥3 toxicity (%)
Ready et al.,[25] 2018	II	Nivolumab 3 mg/kg	109	11.9	1.4	5.6	11.9
Hellman et al.,[27] 2018	Retrospective	Nivolumab 3 mg/kg	TMB Low 42	4.8	1.3	3.1	NR
			TMB Medium 44	6.8	1.3	3.9	NR
			TMB High 47	21.3	1.4	5.4	NR
Hellman et al.,[27] 2018	Retrospective	Nivolumab 1 mg/kg + I 3 mg/kg	TMB Low 27	22.2	1.5	3.4	NR
			TMB Medium 25	16.0	1.3	3.6	NR
			TMB High 26	46.2	7.8	22.0	NR
Chung et al.,[28] 2018	II	Pembrolizumab	107	18.7	2.0	9.1	4.6
Goldman et al.,[29] 2018	I/II	Durvalumab	21	9.5	1.5	4.8	0.0
Pujol et al.,[30] 2018	IIR	Atezolizumab	49	2.3	1.4	NR	8.4
		Topotecan	24	9.5	4.2	NR	0.0

I: ipilimumab; IIR: Phase II randomised; NR: not reported; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; pts: patients; SCLC: small cell lung cancer; TMB: tumor mutational burden.

*Only patients with PD-L1 > 1%.

ONGOING TRIALS

To date, only results from trials addressing first-line ED-SCLC or second and further-line treatment and containing checkpoint inhibitors are available. No data addressing LD-SCLC are available yet. Several ongoing trials are investigating immunotherapy in the first-line setting for ED-SCLC patients. Part E of the Phase I KEYNOTE-011 study is evaluating the safety and efficacy of pembrolizumab in combination with platinum/etoposide.[36] The Phase II randomised multicentre open-label REACTION trial is assessing first-line platinum/etoposide with or without pembrolizumab.[37] KEYNOTE-604, a Phase III randomised, double-blind study, is enrolling 430 patients to platinum/etoposide plus pembrolizumab or placebo. Co-primary endpoints are PFS and OS.[38] CASPIAN is a Phase III, open-label study which completed the accrual of 795 patients who were randomised to either platinum-etoposide versus platinum-etoposide + durvalumab, a human immunoglobulin G1 kappa monoclonal antibody against PD-L1, versus platinum-etoposide + durvalumab and tremelimumab, a fully human monoclonal antibody against CTLA-4. The primary endpoint is OS and the results are still pending.[39] CheckMate-451 is a Phase III trial enrolling 810 ED-SCLC patients who did not progress after completion of first-line platinum-etoposide to receive maintenance nivolumab versus nivolumab + ipilimumab versus placebo. Co-primary endpoints are PFS and OS.[40] A Phase I/II study is investigating the combination of nivolumab plus ipilimumab with thoracic radiotherapy (30 Gy in 10 fractions) following standard platinum-based chemotherapy. The study will enrol 52 ED-SCLC patients over

two parts. Part I of the study will establish the recommended Phase II dose of immunotherapy when combined with thoracic radiotherapy, whereas Part II will estimate the 6-month PFS.[41]

Several trials are ongoing in LD-SCLC patients. Pembrolizumab and concurrent thoracic radiotherapy with or without chemotherapy are being investigated within a Phase I single-centre study. The aim is to determine the maximum tolerated pembrolizumab dose given in combination with radiotherapy in 80 patients. Secondary endpoints are PFS and safety.[42] The randomised, Phase II, open-label STIMULI trial is evaluating the consolidation of nivolumab and ipilimumab following completion of chemo-radiotherapy. Patients will be randomised to an induction phase of nivolumab 1 mg/kg + ipilimumab 3 mg/kg, every 3 weeks for 4 cycles, followed by a maintenance phase (nivolumab 240 mg every 2 weeks for 12 months) or observation. Co-primary endpoints are OS and PFS.[43]

In second or later-line SCLC management, several immunotherapy trials are ongoing. A multicentre, randomised, open-label Phase II study is enrolling 98 patients to pembrolizumab versus topotecan. Although PD-L1 expression determined at baseline is mandatory, the enrolment will occur regardless of PD-L1 status. Crossover to pembrolizumab is allowed in the topotecan arm at progression. The primary endpoint is PFS.[44]

Table 3: Characteristics of the main ongoing trials in SCLC patients.

ID	Phase of study	Title	Treatment	Line of therapy	Primary endpoint	Status
NCT01840579[36]	I	Study of pembrolizumab (MK-3475) monotherapy in advanced solid tumours and pembrolizumab combination therapy in advanced non-SCLC/ ED-SCLC(MK-3475-011/ KEYNOTE-011)	Part E: pembrolizumab +EP	First	Number of participants experiencing dose-limiting toxicities	Active, not recruiting
NCT02580994[37]	IIR	A Phase II study of etoposide and cis/ carboplatin with or without pembrolizumab in untreated extensive SCLC (REACTION)	Pembrolizumab +EP versus EP	First	PFS	Recruiting
NCT03066778[38]	III	A Phase III randomised, double-blind, placebo-controlled trial of pembrolizumab (MK-3475/SCH900475) in combination with etoposide/platinum (cisplatin or carboplatin) for the first-line treatment of subjects with extensive stage SCLC (KEYNOTE-604)	Pembrolizumab +EP versus PLB+EP	First	PFS, OS	Active, not recruiting
NCT03043872[39]	III	A Phase III, randomised, multicentre, open-label, comparative study to determine the efficacy of durvalumab or durvalumab and tremelimumab in combination with platinum-based chemotherapy for the first-line treatment in patients with ED-SCLC (CASPIAN)	Durvalumab +tremelimumab +EP versus Durvalumab+EP versus EP	First	PFS, OS	Active, not recruiting

Table 3 continued.

ID	Phase of study	Title	Treatment	Line of therapy	Primary endpoint	Status
NCT02538666[40]	III	A randomised, multicentre, double-blind, Phase III study of nivolumab, nivolumab in combination with ipilimumab, or placebo as maintenance therapy in subjects with ED-SCLC after completion of platinum-based first line chemotherapy (CheckMate 451: CHECKpoint Pathway and nivoluMAb Clinical Trial Evaluation 451).	Nivolumab versus Nivolumab+ ipilimumab versus PLB	Maintenance	OS	Active, not recruiting
NCT03043599[41]	I/II	Consolidative ipilimumab and nivolumab with thoracic RT after platinum-based chemotherapy for patients with ED-SCLC	Ipilimumab+ nivolumab+ thoracic RT	Consolidation	Phase I: Confirmation of recommended Phase II dose Phase II: PFS	Active, not recruiting
NCT02402920[42]	I	Phase I trial of MK-3475 and concurrent chemo/ radiation for the elimination of SCLC	Pembrolizumab+ EP+RT	First	Safety of pembrolizumab+ CT/RT for LD-SCLC Safety of pembrolizumab+ RT for ED-SCLC	Recruiting
NCT02046733[43]	IIR	A randomised open-label Phase II trial of consolidation with nivolumab and ipilimumab in limited-stage SCLC after chemo-RT (STIMULI)	Nivolumab+ ipilimumab versus observation	Consolidation	OS, PFS	Recruiting
NCT02963090[44]	IIR	A randomised Phase II study evaluating pembrolizumab vs topotecan in the second-line treatment of patients with SCLC	Topotecan versus Pembrolizumab	Second	PFS	Active, not recruiting
NCT02701400[45]	IIR	A randomised study of tremelimumab plus durvalumab combination with or without radiation in relapsed SCLC	Tremelimumab+ durvalumab versus RT followed by tremelimumab+ durvalumab	Second	ORR, PFS	Recruiting

ED: extensive disease; EP: platinum plus etoposide; IIR: randomised Phase II trial; LD: limited disease; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; PLB: placebo; RT: radiotherapy; SCLC: small cell lung cancer.

Another randomised Phase II trial is currently enrolling patients with progressed SCLC to durvalumab plus tremelimumab with or without radiotherapy. Co-primary endpoints are PFS and ORR.[45] Table 3 summarises the main ongoing trials in SCLC patients.

CONCLUSION

Immunotherapy through the administration of anti-CTLA-4 and anti-PD-1/PD-L1 agents, alone or in combination with first-line chemotherapy or in relapsed SCLC, may become a new option for the management of SCLC. To date, nivolumab has already been approved by the FDA for the third-line treatment of SCLC.[26] Moreover, the combination of atezolizumab plus carboplatin plus etoposide improved the OS of SCLC patients in first-line setting.[20] Furthermore, several clinical trials investigating checkpoint inhibitors are still ongoing. Moreover, to optimise the results of immunotherapy the selection of patients based on immune biomarkers is of paramount importance.

In addition, further immune checkpoint inhibitors directed against other targets, such as the killer-cell immunoglobulin-like receptor and lymphocyte-activation gene-3, are in clinical development.[16]

In the next few years, there will be an increasing amount of new data produced through immunotherapy research, and hopefully the research community's high expectations will be met. The author further hopes that these results, together with the drugs under development, will offer new and improved treatment options for SCLC patients.

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Non-melanoma skin cancer (NMSC):

Getting to the root of the problem

NMSC is the most common cancer, and its incidence is rising[1]

Studies in Canada, the United States, Switzerland, and Australia have shown that the incidence of NMSC has been increasing at 2% to 8% per year since the 1970s.[2]

Advanced NMSC can be debilitating, with significant psychosocial and functional impacts on patients[3,4]

Typically, NMSC is curable with complete surgical excision. However, the cosmetic and functional results of treatment for advanced disease, such as scarring and disfigurement from surgery or radiation, can have a profound impact on patients.[1,3,4]

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Sanofi Genzyme and Regeneron are committed to providing resources to better understand the incidence, identification, and treatment of NMSC and to research the unmet needs of patients with this disease.

Chemokine Pathway Can Be the Potential Therapeutic Target for Hypertrophic Scar

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Abstract

Hypertrophic scar (HTS) is a dermal form of fibroproliferative disease that develops after deep burns, skin injuries, and surgical wounds. HTS is defined as a visible, elevated scar that does not spread into surrounding tissues and often regresses. HTS often leads to physical and psychological issues for patients. Cellular and molecular agents, such as chemokines, bone marrow derived stem cells, TGF-β, fibroblast heterogeneity, toll-like receptor 4, and small leucine-rich proteoglycans, have been known to play an important role in wound healing and abnormal scar formation. Targeting these agents could have therapeutic potential for the treatment of pathological scars. HTS often causes contracture deformities, aesthetic disfigurement, and prolonged periods of hospitalisation and rehabilitation, leading to psychological complications. Numerous treatments have been described for HTS, but the optimal treatment has not yet been established. Chemokines are implicated in all stages of wound healing, but hyperactivity of these agents leads to excessive scarring. A better understanding of the mechanisms of chemokine action, such as the role of the signalling agents in wound healing and abnormal scar formation, will help to establish more effective therapeutic strategies for HTS.

INTRODUCTION

Hypertrophic scar (HTS) is a dermal form of fibroproliferative disease that develops after deep burns, skin injuries, and surgical wounds. HTS is defined as a visible, elevated scar that does not spread into surrounding tissues. It often causes contracture deformities, aesthetic disfigurement, and a prolonged period of hospitalisation and rehabilitation, which can lead to psychological complications.[1,2] Numerous treatments have been described for HTS, but the optimal treatment has not yet been established.

Cellular and molecular factors, such as chemokines, bone marrow derived stem cells (BMSC), TGF-β, fibroblast heterogeneity, toll-like receptor 4 (TLR4), and small leucine-rich proteoglycans (SLRP), are known to play important roles in wound healing and abnormal scar formation; therefore, targeting these agents has therapeutic potential for pathological scars. Chemokines in particular are implicated in all stages of wound healing and hyperactivity leads to excessive scarring. Having a better understanding of the mechanisms of chemokine pathways in wound healing and abnormal scar formation will help to establish more effective therapeutic strategies for HTS.

This review will summarise cellular and molecular mechanisms in wound healing and HTS formation and describe the possibility of targeting the chemokine pathways as a therapeutic approach for HTS.

WOUND HEALING AND HYPERTROPHIC SCAR FORMATION

Normal wound healing consists of four overlapping phases: haemostasis, inflammation, proliferation, and remodelling. Wound healing requires complex interactions among keratinocytes, fibroblasts, the extracellular matrix (ECM), and stem cell populations within the basal epidermis, appendages, and underlying mesenchyme.[3] These interactions are coordinated and regulated by a variety of molecules, including growth factors, cytokines, and chemokines.

The inflammatory phase presents as erythema, swelling, fever, and pain around wound sites, which usually lasts 2–4 days after injury. During inflammation, leukocytes leave adjacent blood vessels and migrate to the wound sites, where the cells facilitate the removal of both micro-organisms and cellular debris and secrete a plethora of cytokines that are critical for proper healing. This phase of healing is mediated via growth factors, cytokines, and chemokines, which induce the recruitment of different cell types to the wound sites to promote wound healing.[4]

The proliferation phase involves fibroplasia, angiogenesis, and the formation of the ECM and the granulation tissue. Fibroplasia is the process whereby fibroblasts proliferate in the wound sites and excrete glycoprotein and collagen, which combine to form the ECM. Angiogenesis is essential to replace damaged capillaries and restore the supply of oxygen, blood constituents, and nutrients to wounded tissue; this process drives the return to normoxia and promotes fibroplasia.[5] Angiogenesis simultaneously takes place, which involves the migration of endothelial cells to the wounded area. During the formation of granulation tissue, fibroblasts are activated and differentiate into myofibroblasts, which are highly contractile cells that produce an abundance of ECM proteins. Myofibroblasts contribute to wound contraction, which is an essential process in wound healing.

The remodelling phase begins 2–3 weeks after injury and can last for a year or more. During the remodelling phase, granulation tissue is converted into a mature scar. Type III collagen, the primary component of the granulation tissue, is replaced by type I collagen, which is the primary structural component of the dermis.[6] Disorganised collagen fibres are rearranged, cross-linked, and aligned along tension lines. Hypercellularity in remodelling wounds is reduced by apoptosis of residual inflammatory cells, vascular cells, and myofibroblasts.[7]

Pathological wound healing, mainly caused by chronic inflammation or infection, can lead to fibroproliferative disorders, such as HTS and keloids.[8] Increased vascular permeability, which is the result of dilatation of the gaps between the endothelial cells of the vascular wall, allows inflammatory cells to migrate into the wound site. This endothelial function is intensified by genetic mutation, systemic factors, and local factors, such as skin tension. Endothelial dysfunction increases the number of inflammatory cells and the levels of many soluble inflammatory factors that can enter the wound site, thereby increasing local inflammation.[9] In some excess healing conditions, the myofibroblasts fail to undergo apoptosis, leading to their prolonged presence in the wound and production of excessive ECM, resulting in fibrosis and abnormal scar formation.[10]

EPIDEMIOLOGY

It is estimated that HTS develops in up to 67% of burn patients with major burns, as defined by the American Burn Association (ABA). The rate is higher in non-white patients and the lowest incidence of HTS is found in patients with albinism.[11] Children have a higher rate of HTS formation than adults.[12]

AETIOLOGY

An analysis of surgical burn treatment showed that burn depth, type of skin graft; delayed wound healing caused by infection or prolonged inflammation, and wound tension are risk factors for HTS.[13,14] BMI, non-Caucasian ethnicity, and scar related factors are positively associated with HTS formation. On the other hand, antihypertensive therapeutics and factors

influencing erythropoiesis were negatively associated with HTS formation in patients who underwent elective cardiothoracic surgery.[15]

CLINICAL SYMPTOMS

Morphologically, HTS presents as a red, hyperaemic, elevated, firm scar and pruritic lesions that have an abnormal texture, which have lost the pliable and elastic attributes of healthy skin. HTS often causes contracture deformities, aesthetic disfigurement, a prolonged period of hospitalisation and rehabilitation, and psychological issues[2,8] (Figure 1).

HISTOLOGY

The main histological features of HTS are a thicker epidermis and dermis; a lack of rete ridges in the epidermis; increased cell proliferation; excess blood vessel formation; and an atypical ECM, featuring increased collagen deposition and thin disorganised collagen fibres in the dermis (Figure 2).[16] Collagen bundles contain a greater amount of type III collagen than normal skin and are orientated parallel to the epidermal surface. Fibroblasts and myofibroblasts play essential roles in fibrotic diseases due to the ability of these cells to generate excessive collagen in abnormal wounds; additionally, fibroblasts are the primary contributors of collagen deposition at the wound site. Ultrastructural nodules and large numbers of myofibroblasts expressing alpha smooth muscle actin are seen in the histology of HTS.[16]

CURRENT TREATMENTS

Numerous treatments have been described for HTS. Among the therapeutic options are surgical excision, pressure therapy, intralesional interferon, corticosteroids, bleomycin, 5-fluorouracil injections, combination of cryotherapy with intralesional triamcinolone acetonide, silicone gel sheeting, onion extract and heparin gel administration, and laser therapy.[17] Currently, the most widely accepted therapeutic modalities are surgery plus adjuvants, such as intralesional steroids, silicone gel sheeting, pressure, and radiotherapy. Despite these treatments, HTS remains difficult to manage and there is no universally accepted treatment regimen.[17]

BONE MARROW DERIVED STEM CELLS IN HYPERTROPHIC SCARRING

BMSC, such as mesenchymal stem cells, endothelial progenitor cells, and fibrocytes, are involved in the wound healing processes, contributing to skin cells or releasing regulatory cytokines.[18] By co-culturing layered fibroblasts with bone marrow derived-mesenchymal stem cells (BM-MSC), the BM-MSC enhanced the fibrotic behaviour of deep dermal fibroblasts.[19] Otherwise, BM-MSC conditioned medium decreased expression of profibrotic genes, including connective tissue growth factor, plasminogen activator inhibitor-1, TGF-β1, and TGF-β2 in HTS fibroblasts. In contrast, the expression of antifibrotic genes, such as *TGF-β3* and *DCN*, were substantially enhanced.[20]

Hypertrophic scars

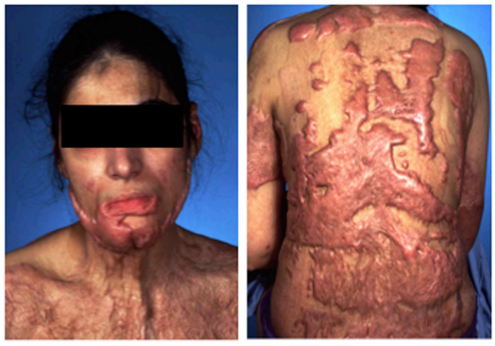

Figure 1: Hypertrophic scars in a 28-year-old woman with after a burn injury.

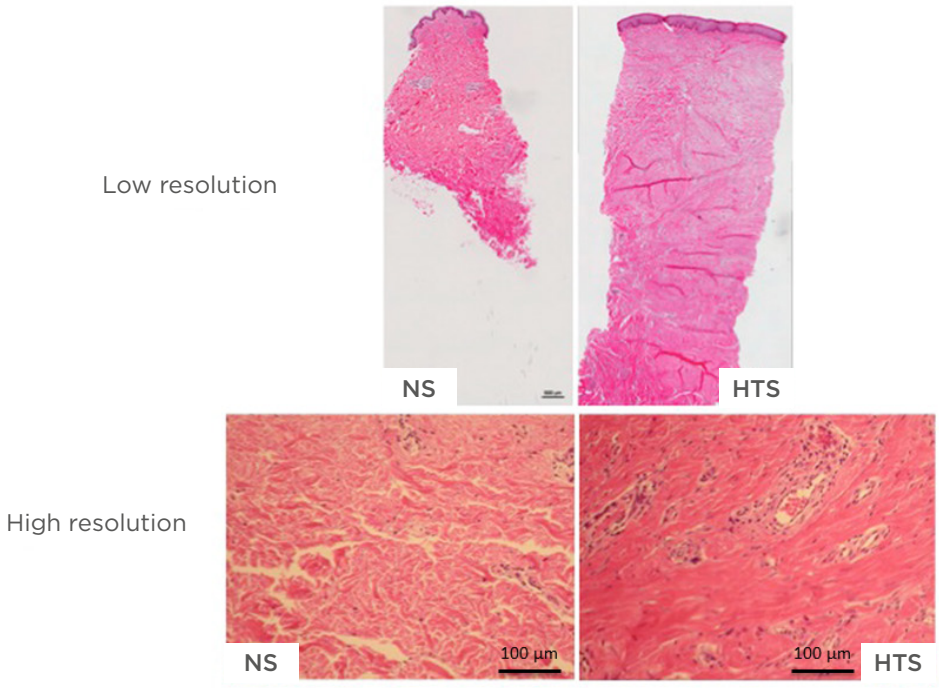

Figure 2: High and low-resolution imaging of normal skin and hypertrophic scarring.

The main histological features of hypertrophic scarring are a thicker epidermis and dermis, lack of rete ridges in the epidermis, increased cell proliferation, excess blood vessel formation, and atypical ECM, such as collagen deposition, and thin disorganised collagen fibres in the dermis. Low resolution: 10x magnification; high resolution: 40x magnification.

ECM: extracellular matrix; HTS: hypertrophic scarring; NS: normal skin.

BM-MSC enhance wound healing and prevent HTS formation via inflammatory regulation, differentiation, and angiogenesis and/or release of paracrine factors.[19] These studies suggest the involvement of BM-MSC in the pathogenesis of hypertrophic scarring and suggest their therapeutic potential for HTS.

TGF-β IN HYPERTROPHIC SCARRING

TGF-β has recently been shown to be a key factor in modulating contraction in normal skin fibroblasts.[6] There are three mammalian isoforms of the growth factor: TGF-β1, TGF-β2, and TGF-β3; each of the isoforms have different effects on the same cells. TGF-β strongly promotes the chemotaxis of fibroblasts to the site of inflammation to produce ECM proteins. Myofibroblasts are an important source of ECM in the development of fibrosis. The transition from fibroblasts to myofibroblasts is influenced by TGF-β1, which is a profibrotic mediator and regulator of the wound healing processes at cellular level, including the regulation of proliferation, differentiation, migration, cell survival, and angiogenesis.[6] Dysregulation of TGF-β production or activity can cause abnormal fibrosis. Fetal wounds, which heal without scarring, contain lower levels of TGF-β than adult wounds. HTS tissue expressed five times as much TGF-β1 mRNA as normal skin tissue. *In vitro* cultured fibroblasts derived from the HTS also expressed TGF-β1 mRNA at a level significantly higher than that of the normal fibroblasts.[21] In fetuses that heal without a scar, the ratio of TGF-β3 to the TGF-β1 and TGF-β2 isoforms is high, while in wound healing, the ratio of TGF-β3 to TGF-β1 and TGF-β2 is low, with expression of TGF-β3 emerging at a later stage of the healing process.[22] TGF-β3 is a potential mediator of scar reduction and improvement, with the isoform having been used in a surgical model of cleft lip in mouse and sheep fetuses.[23] Human recombinant TGF-β3 (avotermin) was shown to promote the regeneration of normal skin and improve scar

appearance in Phase I and II trials.[24] Both *in vitro* and *in vivo* models have demonstrated that avotermin affects a number of processes, including the inflammatory response, cell migration, and protein deposition in the ECM, which collectively result in scar improvement. However, avotermin failed in Phase III clinical trials.[24]

Bone morphogenetic proteins and activins belong to the TGF-β superfamily and are involved in almost every phase of wound healing. These molecules promote epidermal hyperthickening and re-epithelialisation, keratinocyte proliferation, granulation tissue formation, epithelial to mesenchymal transition, and ECM production. Activins are upregulated after skin injury and production of these proteins is increased in HTS.[25]

FIBROBLAST HETEROGENEITY IN HYPERTROPHIC SCARRING

Fibroblasts synthesise the ECM of connective tissue and play an essential role in maintaining the structural integrity of most tissues. Fibroblasts isolated from different tissues in the body belong to morphologically and functionally heterogeneous subpopulations. Furthermore, these differences seem to be dictated by the local biological and physical microenvironment the fibroblasts reside in. *In vitro*, deep dermal fibroblasts were found to contain higher levels of mRNA of type 1 collagen, alpha smooth muscle actin, TGF-β, stromal cell-derived factor 1, and tissue inhibitor of metalloproteinase 1, an inhibitor of collagenase.[19] Furthermore, deep dermal fibroblasts express a higher number of TGF-β type II receptors; have lower collagenase activity; and produce more fibrotic factors, including TGF-β1, connective tissue growth factor, osteopontin, biglycan, and versican, and less antifibrotic SLRP.[19] Fibroblast heterogeneity is necessary for scarless wound healing, complete restoration of native tissue architecture in the fetus and oral mucosa, and excessive scar formation, such as that seen in HTS and keloids.[3]

TOLL-LIKE RECEPTORS IN HYPERTROPHIC SCARRING

It is understood that prolonged inflammation contributes to HTS formation. Fibroblasts regulate immune and inflammatory responses through TLR4, which is activated by lipopolysaccharide through adaptor molecules, leading to NFκB and mitogen-activated protein kinase activation, cytokine gene transcription, and co-stimulatory molecule expression, which results in inflammation. Dermal fibroblasts from HTS express TLR, and the associated intracellular signalling molecules express at a higher level than normal. As a result, lipopolysaccharide-stimulated fibroblasts express proinflammatory cytokines, including prostaglandin E2, IL-6, IL-8, and monocyte chemoattractant protein-1, which cause persistent inflammation in burn-injured tissue, thus contributing to HTS development.[26]

EXTRACELLULAR MATRIX COMPONENTS IN HYPERTROPHIC SCARRING

The ECM is a non-cellular, three-dimensional, macromolecular network composed of collagens, elastin, fibronectin, laminins, proteoglycans/glycosaminoglycans, and several other glycoproteins. The ECM has many functions, such as storage and delivery of growth factors and cytokines, tissue repair, and various other physiological functions. Abnormal ECM reconstruction leads to HTS.[27] The breakdown of the ECM is an essential component of wound healing and scar formation. Matrix metalloproteinases (MMP), classified by their substrate specificity, catalyse the hydrolysis of major ECM molecules. Furthermore, MMP affect many biological functions by regulating growth factors and their receptors, cytokines and chemokines, cell surface proteoglycans, and other enzymes. MMP are secreted by keratinocytes, fibroblasts, and inflammatory cells during the wound healing process. Mice deficient in MMP, including MMP-3, MMP-8, and MMP-14, showed delayed or impaired wound healing. In HTS, MMP-1, MMP-2, and MMP-9 are decreased and tissue inhibitors of MMP-1 are increased.[27]

SLRP, such as decorin, biglycan, fibromodulin, and lumican, are ECM molecules that bind to type I collagen to regulate collagen fibrillogenesis and inhibit TGF-β1 fibrogenic activity.[6,28] Under healthy conditions, higher levels of decorin and fibromodulin have been found in the superficial layers of the skin

compared with levels recorded in the deeper dermal layers. There is an increased expression of biglycan, fibromodulin, and lumican in the basement membrane and around basal epithelial cells. These proteoglycans are absent or weakly expressed in HTS compared to normal skin. Decorin knock-out mice showed a significant increase in the number of fibroblasts in their periodontal ligaments compared with wild-type mice. Decorin deficiency leads to impaired angiogenesis in injured mouse corneas. These findings suggest that down-regulation of SLRP after deep injuries to the skin play an important role in the development of HTS.[28]

THE ROLES OF THE CHEMOKINE PATHWAY IN HYPERTROPHIC SCARRING

In recent years, many scientific research papers have described the roles of chemokines in the wound healing processes but also various other biological processes and diseases, such as HIV-1, cardiovascular diseases, cancer, atherosclerosis, inflammatory bowel diseases, and rheumatoid arthritis.[29-31] Chemokines are a family of small chemotactic cytokines, 8–10 KDa in size, which have been classified into four main subfamilies: C (XCL), CC (CCL), CXC (CXCL), and CX3C (CX3CL) chemokines. The chemokines play critical roles in many basic biological processes, including leukocyte trafficking and homing, tumourigenesis and metastasis, inflammation, autoimmune response, and viral infection.[29-31]

The normal wound healing processes are regulated by numerous bloodborne cells; the recruitment of these cells is tightly regulated by chemokines. The chemokines are important modulators of each phase in human wound healing.[4,29,32] A lack of regulation of the chemokine network can result in chronic inflammation, dysregulation of vessel development, and establishment of a chronic environment that leads to impaired healing, such as HTS, keloids, scleroderma, psoriasis, and various types of fibrosis and chronic wounds.[10] HTS is hypercellular due to increased numbers of fibroblasts and the recruitment of peripheral non-haematopoietic cells. The recruitment of bloodborne cells suggests that chemokines may have roles during HTS development.[32]

After injury, the haemostasis phase starts immediately, and platelets and plasma fibronectin are released along with prothrombin to form a clot. During this phase, CXCL4 plays an important role. CXCL4 has weak chemotactic potency, but it is strongly involved in angiogenesis, haematopoiesis suppression, inhibition of collagenase activity, and accumulation of deleterious lipoproteins at sites of vascular injury. CXCL4 inhibits local antithrombin III activity and promotes coagulation by neutralisation of heparin-like molecules on the endothelial surface of blood vessels.[4,29,32]

The inflammatory phase is mediated via growth factors, cytokines, and chemokines, which induce the recruitment of different cell types to the wound sites to promote healing.[10] Recruitment of proinflammatory cells, such as neutrophils and macrophages derived from monocytes, to the injured sites is necessary to remove debris and kill bacteria through phagocytosis and free radical production. These leukocytes are mainly directed by CXC chemokines, such as CXCL1, CXCL5, and CXCL8, that are stored in blood platelets and are immediately released upon cell activation. CXCL8 was the first chemokine identified as a mediator of the directional migration of leukocytes to sites of inflammation and injury. CXCL8 also activates endothelial permeability through both modulation of adherens junction endothelial cell adhesion and subsequent cell contractions that promote leukocyte diapedesis from the circulation.[30] In humans, CXCL8 is one of the main chemoattractants for neutrophils through both the CXCR1 and CXCR2 receptors and stimulates neutrophil activation through CXCR1.[31,33]

Neutrophil chemotaxis is followed by monocyte chemotaxis to the wound sites, primarily via the CCL2 protein: monocyte chemoattractant protein-1. Monocyte chemoattractant protein-1 is released by neutrophils during the early stages of wound healing, by the monocytes themselves, and by keratinocytes during the later stages of wound healing.[34] In the wound sites, monocytes differentiate into macrophages, which phagocytose apoptotic neutrophils and other dead cells, and secrete cytokines, growth factors, and chemokines that promote the latter stages of inflammation and wound repair. Macrophages also stimulate angiogenesis and fibroplasia and ECM production.[35]

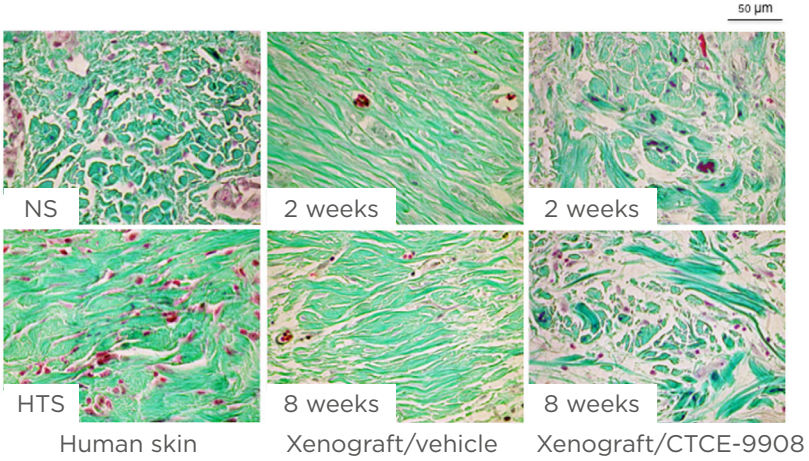

Figure 3: Collagen fibre morphology in the dermis of xenografts.

Representative Masson's trichrome stain images for collagen fibres in the dermis of xenografts. Masson's trichrome stain was performed in paraffin-embedded human normal skin, human hypertrophic scarring, and xenograft biopsies collected from mice treated with CTCE-9908 or vehicle at 2 and 8 weeks after grafting, which stains keratin red, collagen green, cell cytoplasm pink, and cell nuclei dark brown.

HTS: hypertrophic scarring; NS: normal skin.

Re-epithelialisation occurs during inflammation and the formation of the granulation tissue. The process involves keratinocyte migration and proliferation to cover the wound area. CXCL11 and its receptor CXCR3 are expressed by basal keratinocytes during re-epithelialisation and promote re-epithelialisation as a mediator of epidermal-dermal communication during wound repair.[4,36] CXCR3 knock-out mice showed delayed re-epithelialisation and basement membrane regeneration after wounding.[36]

CCL2 binds CCR2 presented on endothelial cells and has a role in angiogenesis. CCL2 is produced by a number of cell types, including fibroblasts and endothelial, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells. This pathway mediates neovascularisation by affecting membrane type 1-MMP.[37] This pathway also regulates migration and infiltration of monocytes into the foci of active inflammation in the wound healing process,[38] and has a pathogenic role in the induction of multiple types of fibrosis, such as HTS, keloids, scleroderma, liver cirrhosis, atherosclerosis, pulmonary fibrosis, renal fibrosis, and colon fibrosis. CCL2 knock-out mice form minimal scars, indicating that the inflammatory chemokine pathways are a major mechanism by which focal adhesion kinase mechanotransduction induces fibrosis.[39]

CCL5 (regulated on activation, normal T cell expressed and secreted) interaction with CCR5 also has a role in angiogenesis. CCL5 increases vascular endothelial growth factor expression and production of human osteosarcoma cells.[40] CCR5 is expressed on the surface of various immune cells, including macrophages, monocytes, microglia, dendritic cells, and active memory CD4+ T cells. Endothelial progenitor cell recruitment to the wound site is regulated by CCR5.[41] In mice, *CCR5* gene expression and its ligands are upregulated at the wound site and the CCR5 protein is exclusively detected in macrophages and endothelial cells. Deficiency of CCR5 reduced endothelial progenitor cell recruitment and attenuated neovascularisation and collagen production in skin wounds. This resulted in delayed wound healing.[41]

CXCL12 (stromal cell-derived factor-1) is expressed in the epidermis, dermis, hair follicles, blood vessels, and sweat glands. CXCL12 specifically binds to CXCR4 receptors on endothelial cells and induces endothelial cell chemotaxis, which is important for vascularisation. CXCL12 is involved in the homing of BMSC to the wound site during skeletal,

myocardial, vascular, lung, and skin wound repair via CXCR4.[29]

The CXCL12–CXCR4 pathway is involved in the development of HTS by promoting the migration of activated CD14+ CXCR4+ cells from the bloodstream to wound sites, where these differentiate into fibrocytes and myofibroblasts, contributing to the development of HTS.[42] Local dermal fibroblasts are activated by inflammatory signals after injury and produce CXCL12, which attracts CXCR4-expressing monocytes to the wound site from the systemic circulation. The CXCL12–CXCR4 pathway is upregulated during wound healing in burn patients and in HTS tissue compared with normal skin.[42] Blocking the CXCL12–CXCR4 pathway by CTCE-9908 (a CXCR4 antagonist) minimised HTS formation in mouse model. CTCE-9908 reduced the accumulation of macrophages and myofibroblasts, enhanced the remodelling of collagen fibres, and downregulated the gene and protein expression of fibrotic growth factors in the skin grafts[43] (Figure 3). The endogenous stem cell mobilisation produced by AMD3100 (CXCR4 antagonist) and low-dose tacrolimus reduced the time to complete healing of full-thickness wounds by 25% in mice and resulted in fewer scars.[44]

In the remodelling phase, reduction in hypercellularity is controlled by the interaction of CXCL10 and CXCL11, which are respectively produced by maturing endothelium and keratinocytes, with the CXCR3 receptor.[4,37] Stimulation of CXCR3 signalling converts fibroblasts from a migratory to a contractile state after an increase of mature dermal collagen fibres, increases keratinocyte migration by activation of m-calpain, and inhibits endothelial cell migration and proliferation.[4] In an *in vivo* study,[45] full-thickness excisional wounds in CXCR3 knock-out mice healed with dermal hypercellularity and presented with immature matrix components. They also presented with poor remodelling and reorganisation of collagen, which resulted in a healed dermis that lacked tensile strength. It is hypothesised that hypertrophic scarring results from the fibroplasia and its overproduction of ECM, secondary to abnormalities in epidermal-dermal communication. Signalling through the CXCR3 receptor plays a major role in wound maturation; the absence of this signalling system results in an immature and hypercellular dermis, and HTS characterised by ongoing wound regeneration, cellular proliferation, and scars in which immature matrix components are undergoing increased turnover resulting in a chronic inflammatory process.[45]

POTENTIAL THERAPEUTIC STRATEGY FOR HYPERTROPHIC SCARRING IN THE FUTURE: TARGETING CHEMOKINE PATHWAYS

Chemokines are essential for wound healing and scar formation by coagulation, leukocyte infiltration, re-epithelialisation, angiogenesis, stem cell recruitment, collagen reorganisation, and lymphocyte-mediated immune response. After dermal injury, bloodborne cells are recruited to the wound sites from systemic circulation by chemokines, such as CXCL12, which is released from dermal fibroblasts. These cells are considered as progenitor cells of macrophages and fibrocytes and contribute to not only wound healing but also hypertrophic scarring. The regulation of these processes by inhibition of chemokine pathways, including the CXCL12–CXCR4, CCL2–CCR2, and CCL5–CCR5 mechanisms, has the potential to reduce hypertrophic scarring. These pathways are a potential therapeutic target for HTS.

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A New Twist to Ibuprofen: Alternative Action in Alternative Splicing

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Abstract

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) and is a widely used medication. One indication of NSAID use is long-term chemoprevention to decrease the risk of developing various types of cancer, in particular colorectal cancer. The molecular mechanism behind the antitumour properties of NSAID has been largely attributed to inhibition of the enzyme cyclooxygenase. In this review article, the authors highlight that additional mechanisms of NSAID, especially ibuprofen, action exist that are related to cell signalling and the modulation of gene expression, including alternative splicing. For example, the authors describe how ibuprofen inhibits expression of the tumour-related splicing variant RAC1b, which is overexpressed in a specific subset of colorectal tumours. The mechanism involves changes in the phosphorylation of splicing factors that regulate this alternative splicing event. According to recent studies, ibuprofen interferes with signal transmission via protein kinases, a process which is frequently altered in cancer cells.

INTRODUCTION

Ibuprofen belongs to the group of nonsteroidal anti-inflammatory drugs (NSAID) used to treat diverse inflammatory processes, pain, or fever. The mechanism underlying the effects of ibuprofen stems from the inhibition of cyclooxygenase (COX) activity, which is required for prostaglandin (PG) synthesis.[1] PG are produced from plasma membrane-derived arachidonic acid and local PG production has hormone-like effects. Two COX isoforms are expressed in human tissues: the constitutively expressed COX-1 isoform exists in most tissues while the COX-2 isoform is strongly induced during the inflammatory response, including pathological conditions of chronic inflammation and colon cancer.[2] Among

different COX-2-derived products, the highest PGE2 levels are found in tumours and affect various processes, including cell proliferation and apoptosis.[3] In normal physiology, PGE2 plays a role in the maintenance of the gastrointestinal mucosa regulating processes, such as mucus secretion and blood vessel dilation.[4] Thus, prolonged NSAID treatment can lead to side effects, including intestinal bleeding. Most NSAID, including ibuprofen, inhibit both COX isoforms.

Prophylactic use of NSAID has been documented to reduce the risk of dying from colorectal cancer.[5-9] For example, a 300 mg daily dose of aspirin over a period of 10 years revealed a statistically significant protective effect.[7,10,11] A similar risk reduction was reported with a daily ibuprofen dose of 200 mg[8,11-19] for various tumour types: 51% reduction in risk for colon, 72% for breast, 62% for prostate, and 59% for lung cancer.[19]

HOW DOES IBUPROFEN PREVENT CANCER?

Accumulating evidence has revealed that inflammation promotes tumourigenesis,[20,21] in particular when the tissue is under chronic inflammatory conditions. Within the tumour microenvironment, inflammatory cells exchange signals with tumour cells. Stromal cells secrete survival factors for tumour cells while tumour cells produce cytokines, which trigger the proteolytic remodelling of the extracellular matrix by stromal cells, or the formation of new blood vessels.[20,22] Ibuprofen inhibits COX activity and the subsequent generation of proinflammatory PG; this action is thought to underlie the chemopreventive effect of ibuprofen. PGE2, for example, activates G protein-coupled PGE2 receptors that stimulate various signalling pathways involved in cell proliferation and survival.[23,24]

In this article, the authors review additional mechanisms of action that are independent of COX-2 inhibition with the aim of increasing awareness that the clinical effects of ibuprofen can be mediated by several cellular processes. The presented evidence was retrieved from the PubMed search engine using "ibuprofen AND cancer" as the search term. Studies reporting COX-independent effects, including those conducted in the authors' laboratory, were selected for review.

ADDITIONAL MECHANISMS THROUGH WHICH IBUPROFEN INHIBITS TUMOUR CELLS

In 2015, Matos and Jordan[25] reviewed the treatment of cancer cells with ibuprofen. HCT-116 colorectal cells do not express COX-2, but the treatment with 2 mMol/L ibuprofen produced proapoptotic effects.[26] Ibuprofen at a low concentration of 100 µMol was further identified as a direct and COX-independent ligand of peroxisome proliferator-activated receptor gamma (PPARγ),[27] and was shown to stimulate its nuclear activity in rat models of colon cancer formation.[28] Thus, the proapoptotic action observed for ibuprofen may in part result from PPARγ activation, which leads to the downregulation of the antiapoptotic transcription factor NFκB.[28]

Another COX-independent cellular response following ibuprofen treatment was reported to involve $P75^{NTR}$, a member of the TNF receptor superfamily. Treatment of cancer cells with 1 mMol/L ibuprofen resulted in a p38 mitogen-activated protein kinase pathway-dependent stabilisation of $p75^{NTR}$ mRNA stability, leading to increased expression levels[29] and induction of apoptosis and growth suppression.[30]

A similar apoptosis-promoting action was reported in HCT116 cells, when ibuprofen treatment (1.5 mM for 24 hours) was found to sensitise these cells against the TNF-related apoptosis-inducing ligand.[31] The underlying mechanism involved expression of the membrane receptor for TNF-related apoptosis-inducing ligand: death receptor 5, another member of the TNF receptor superfamily.

Ibuprofen treatment (1 mMol/L for 24 hours) was further reported to significantly reduce the nuclear levels of β-catenin in SW480 and DLD-1 colorectal tumour cells. Consistently, the expression of one of its transcriptional targets, the pro-proliferative cyclin D1 gene, was suppressed.[32] Although the underlying mechanism remains to be determined, this effect of ibuprofen seems of special interest for

colorectal cancer prevention because excessive β-catenin signalling can cause inappropriate growth stimulation of colon mucosa stem cells.[33]

Concurrent to the effect on β-catenin signalling, ibuprofen also interfered directly with the NFκB pathway. A rapid effect of ibuprofen treatment observed in cells is the inhibitory phosphorylation of GSK-3β at serine 9.[32] This modification was found to negatively regulate NFκB signalling, at a step downstream of the degradation of its inhibitor protein IkBα, and to suppress the expression of anti-apoptotic NFκB target genes, such as *BCL2* and *BIRC5*.

Other examples for COX-independent effects of 100 µMol ibuprofen include the inhibition of integrin expression in neutrophils[34] or the caspase-mediated release of proinflammatory cytokines in HCT-116 and HeLa cells.[35]

IBUPROFEN, ALTERNATIVE SPLICING, AND CANCER

Cancer cells differ in their gene expression programme from their corresponding differentiated normal cells. Besides transcriptional regulation at gene promoters, the past 15 years have clearly revealed that alternative splicing serves as a significant mechanism for the regulation of gene expression. For example, alternative splicing generates transcript variants that can either be non-functional and rapidly degraded or be translated into protein isoforms with different, sometimes antagonistic, functional properties due to differential use of functional protein domains.[36,37]

Recently, inhibition of the alternative splicing variant RAC1b was identified as another COX-independent effect of ibuprofen.[38] Colon inflammation was shown as one trigger for increased expression of the tumour-related RAC1b protein, a splice variant of the small GTPase RAC1. RAC1b protein contains an additional domain encoded by a 57 base pair-long alternative exon (exon 3b), which confers increased protein activation, generating a hyperactive variant able to stimulate NFκB signalling.[39-42] When colorectal cells were treated with ibuprofen, but not with aspirin or flurbiprofen, both the mRNA and protein levels of RAC1b were markedly reduced *in vitro* and *in vivo*.[38] Whereas many studies on the effect of NSAID on tumour cell viability used concentrations of up to 2 mMol/L,[43] the effect of ibuprofen on alternative splicing of RAC1b was observed at low doses of 100 µMol. Interestingly, ibuprofen inhibited RAC1b-positive HT29 colorectal cells more than normal colonocytes and also affected their growth as subcutaneous tumour xenografts in mice. The inhibitory effect of ibuprofen could be rescued when a splicing-independent RAC1b cDNA sequence was expressed in HT29 cell.[38] This suggests that ibuprofen acts directly on the alternative splicing event.

Another report on the modulation of alternative splicing was obtained when prostate cancer cells received combined treatment of ibuprofen and epigallocatechin-3-gallate (EGCG), a green tea component with anticarcinogenic properties that promotes G0/G1 cell cycle arrest and apoptosis. In this case, the balance between anti and proapoptotic splicing variants of BCL-X and MCL-1 was shifted towards the shorter and proapoptotic BCL-X(S) or MCL-1(S) variants.[44] Although the mechanism was not fully identified, it involves activation of protein phosphatase PP1, which is known to dephosphorylate regulatory proteins involved in pre-mRNA splicing.

MECHANISM OF SPLICING MODULATION BY IBUPROFEN

When protein-coding genes are expressed in human cells, RNA polymerase 2 generates a primary transcript, the pre-mRNA, which contains coding exons separated by intronic sequences. While transcription is ongoing, conserved nucleotide sequences around each exon–intron junction are recognised by the spliceosome, a macromolecular machinery involving five small nuclear ribonucleoprotein particles (U1, U2, U4, U5, and U6 small nuclear ribonucleoprotein),[45,46] which then removes introns during the process of mRNA splicing. The function of the spliceosome is assisted by splice enhancer or silencer elements, short sequences found in exons or introns, which either promote or inhibit productive recognition of a given exon by the spliceosome. Splicing factors recognise these splice enhancer or silencer elements, which mostly belong to the serine and arginine rich protein family or the heterogeneous nuclear ribonucleoproteins. They often act antagonistically, so that the modulation of

binding provides a mechanism that allows inclusion or skipping of alternative exon and thus the generation of variant transcripts. Altogether, the set of splicing factors expressed in a given cell and their relative expression levels in the cell nucleus operate in a combinatorial mode to regulate alternative splicing.

In the case of RAC1b, alternative splicing is regulated by an enhancer element in exon 3b, which is recognised by the splicing factor SRSF1, and an adjacent silencer element recognised by SRSF3.[47]

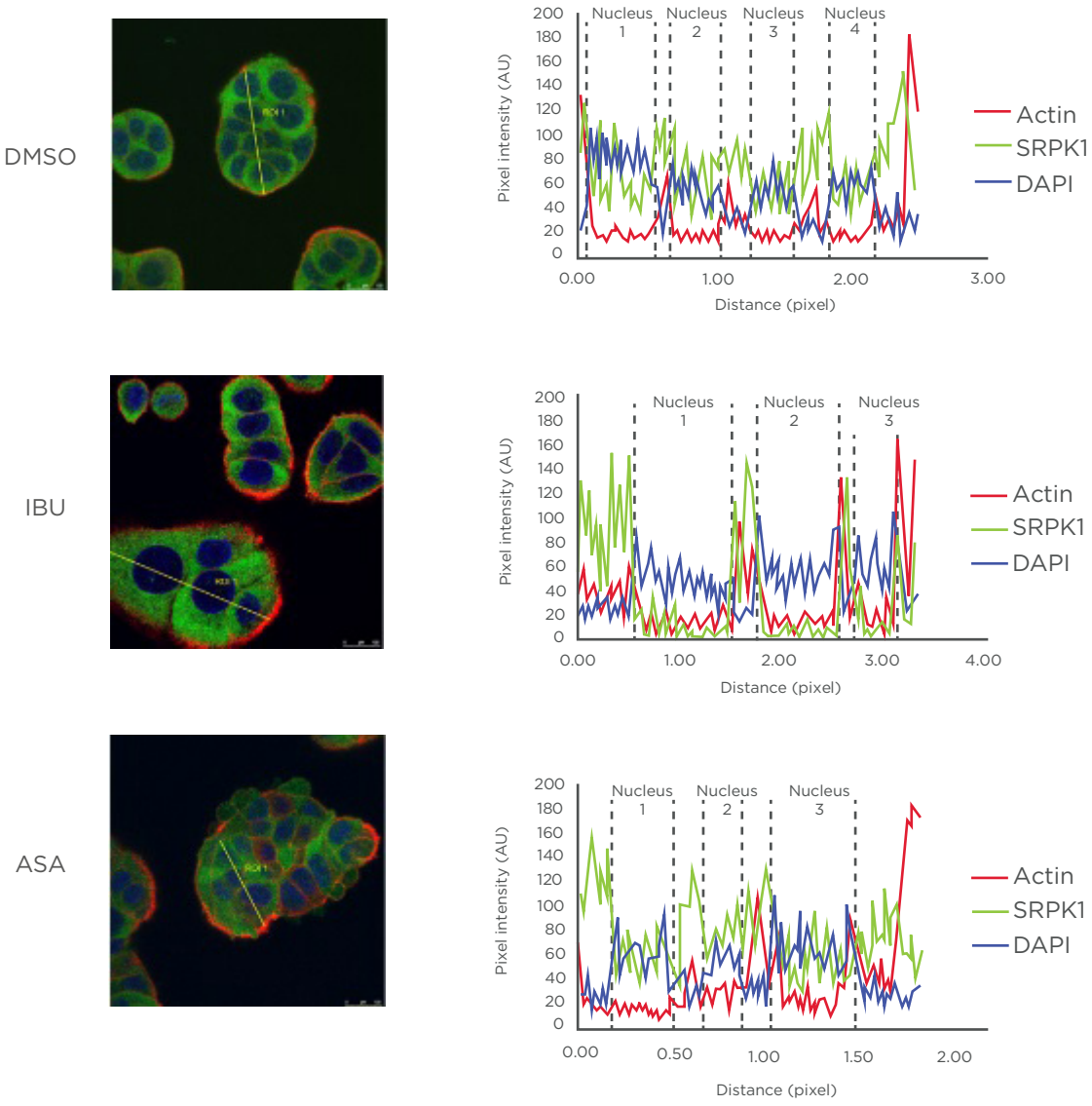

Figure 1: Effect of ibuprofen on subcellular localisation of protein kinase SRPK1.

HT29 colorectal cells were incubated for 48 hours with either a DMSO control solvent, 500 µM ibuprofen, or 500 µM aspirin and then fixed for immunofluorescence microscopy. Shown is the coloured overlay of three confocal immunofluorescence images (left), which detected cell nuclei in blue (DAPI), the localisation of endogenous SRPK1 protein in green (anti-SRPK1, BD Biosciences, San Jose, California, USA), and the actin cytoskeleton in red (Phalloidin-Texas Red). The nucleus and cytoplasm distribution of the three fluorescent signals was analysed along optical sections (yellow lines) across several cells by plotting pixel intensities along the traced path (right graphs). In control and aspirin-treated cells, SRPK1 signals (green) were localised both to the cytosol and the cell nucleus (blue); however, in ibuprofen-treated cells, nuclear signals for SRPK1 are nearly absent.

AU: arbitrary units; ASA: aspirin; DAPI: 4′,6-diamidino-2-phenylindole; DMSO: dimethyl sulfoxide; IBU: ibuprofen; SRPK1: serine/threonine-protein kinase.

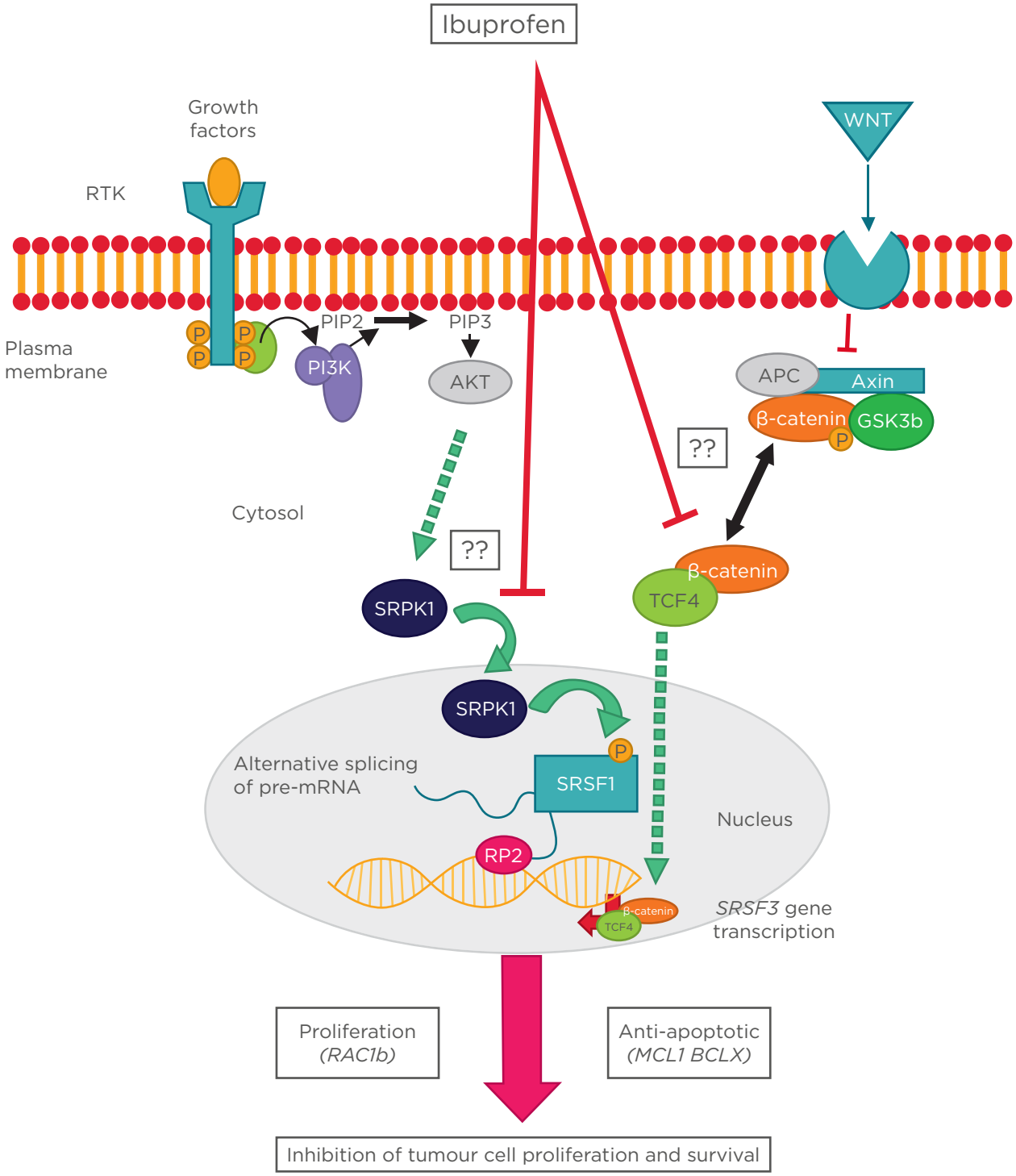

Figure 2: Schematic representation of the cellular pathways linking ibuprofen to the cyclooxygenase-independent modulation of alternative splicing.

Following stimulation of receptor tyrosine kinases, the PI3K becomes activated and leads to phosphorylation of SRPK1, which enters the nucleus and phosphorylates the splicing factor SRSF1. SRSF1 binds to specific recognition motifs on nascent pre-mRNA transcribed by RNA polymerase 2, thus affecting alternative splicing decisions. In a parallel pathway, Wnt ligands stimulate their plasma membrane receptor leading to inhibition of the β-catenin destruction complex and accumulation of a cytosolic β-catenin/TCF4 complex. This complex enters the nucleus and binds to gene promoters, including that of splicing factor SRSF3, the expression levels of which determine the outcome of specific splicing events. Examples of splicing variants affected by these pathways are *RAC1b*, *MCL1*, and *BCLX*. Question marks indicate that the molecular mechanism is still unknown.

AKT: protein kinase B; APC: adenomatous polyposis coli; GSK: glycogen synthase kinase; MCL1: myeloid Cell Leukemia Sequence 1; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-trisphosphate; PI3K: phosphoinositide 3-kinases; RAC1: Ras-related C3 botulinum toxin substrate 1: RP2: RNA polymerase 2; RTK: receptor tyrosine kinases; SRPK1: serine/threonine-protein kinase; SRSF: serine and arginine rich splicing factor; TCF4: transcription factor 4; Wnt: wingless/integrated.

In human colorectal cells, the availability of SRSF1 in the nucleus is the main factor regulating inclusion or skipping of exon 3b.[48]

One mechanism through which ibuprofen does affect alternative splicing in cells is the phosphorylation status of SRSF1. Cell fractionation and immunoblot experiments revealed that ibuprofen treatment caused a reduction in SRSF1 phosphorylation (unpublished data). By contrast, aspirin treatment had no such effect on SRSF1. This showed that the inhibitory effect of ibuprofen on RAC1b splicing involved post-translational regulation of SRSF1 subcellular localisation.[48]

The main protein kinase responsible for SRSF1 phosphorylation is SRPK1, which is found both in the cytoplasm and in the cell nucleus.[49,50] This process is, in part, controlled by growth factor receptor signalling.[51] As shown and described in Figure 1, the authors observed that ibuprofen treatment induced translocation of SRPK1 from the nucleus into the cytoplasm, and this correlated with reduced levels of SRSF1 phosphorylation and RAC1b protein as detected in whole cell lysates by western blot. No such effect was observed when cells were treated with aspirin under the same conditions, underlining the COX-independent action of ibuprofen and the specificity of its effect on splicing factor modulation.

Another mechanism through which ibuprofen can regulate splicing is the transcriptional modulation of splicing factor-encoding genes. The splicing factor SRSF3, for example, was previously described to be a direct transcriptional target for β-catenin/TCF signalling and ibuprofen has been found to downregulate β-catenin/TCF signalling in colorectal cells.[52] A reduction in SRSF3 transcription and the consequent decrease in its nuclear levels will affect a variety of splicing variants.

Further research may unravel that, besides SRSF1 and SRSF3, other splicing factors are also modulated by ibuprofen treatment, either by regulation of their expression levels, their subcellular localisation, or their RNA-binding activity. These effects will most likely also include COX-dependent mechanisms as many of the PGE2 stimulated pathways[23,24] have been described to affect alternative splicing regulation.[53] It can thus be expected that ibuprofen treatment will affect a larger set of alternative splicing events in cancer cells and that these contribute to the described antiproliferative and proapoptotic effects.

CONCLUSION

Although ibuprofen has been used for chemopreventive therapies against cancers in the gastrointestinal tract, our understanding of the molecular mechanisms underlying the antineoplastic activity of ibuprofen is still rudimentary. Recently described cellular pathways linking ibuprofen to the COX-independent modulation of alternative splicing are summarised in Figure 2. A better characterisation of its target molecules and their signalling pathways may provide opportunities for precision medicine approaches in cancer therapy or chemoprevention regimens. For example, the inhibitory effect on alternative splicing of *RAC1b* may benefit a subgroup of colorectal cancer patients characterised by serrated polyp morphology, *BRAF* mutation, and RAC1b overexpression. However, the deregulation of splicing factor SRSF1, which was described in this case, is most likely only the tip of the iceberg. It is now known that deregulation of splicing factors will inevitably affect a network of alternative splicing changes and this can be expected to have significant impact on cancer cell biology. A more systematic study with genome-wide determination of transcriptome changes should clarify the therapeutic opportunities that may arise from the COX-independent action of ibuprofen.

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The Heart of the Matter: A Unique Convergence of Cardiac Neoplasm, Hereditary Nonpolyposis Colorectal Cancer, and Spindle Cell Sarcoma

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Abstract

Primary cardiac tumours are exceedingly unusual and aggressive; they often develop in younger patients and present with advanced disease. The rarity and heterogeneity of primary cardiac tumours challenge the standardisation of therapeutic guidelines. Undifferentiated primary cardiac spindle cell sarcomas, a distinct subset of primary cardiac sarcomas, are especially unique with <20 cases reported worldwide, the majority of which are of left atrial origin. This article presents a review of the aetiology, pathophysiology, and therapy of undifferentiated primary cardiac spindle cell sarcomas. In conjunction, the authors present a unique case of a woman with hereditary nonpolyposis colorectal cancer (Lynch syndrome) who presented with a primary cardiac spindle cell sarcoma of left ventricular origin; this is the first case of this type and location of cardiac tumour reported in a patient with Lynch syndrome.

CARDIAC TUMOURS

Cardiac tumours are rare, with their incidence estimated between 0.0017–0.2800%,[1] and the vast majority are metastatic lesions. Following benign myxomas, malignant primary sarcomas of the heart are the second most common cardiac tumour.[1] Characteristics of malignant cardiac masses include broad-based growth, invasion of surrounding tissue, involvement of more than a single chamber, poor definition of borders, tissue inhomogeneity, large size (>5 cm), and the presence of pericardial or pleural effusion.[2,3] Regardless of benign or malignant status, cardiac tumours are usually asymptomatic until they grow large enough to disrupt valvular function, obstruct a cardiac chamber,[4] or invade the myocardium and conductive tissue to precipitate arrhythmias.[5] Characteristics of cardiac tumours associated with increased survival include left-sided location, the absence of necroses or metastases, and low mitotic count. Notably, age, sex, differentiation, and histology have no effect on prognosis.[6]

The growing incidence of primary cardiac tumours over the past few decades is, in part, a reflection of advancements in cardiac imaging, such as echocardiography, MRI, and multidetector

CT.[7] Echocardiography can help to distinguish between different types of cardiac masses, including thrombi, vegetations, hypertrophy of the interatrial septum, and pericardial cysts.[8] MRI and CT imaging can reveal invasive extracardiac extension of the sarcoma to help determine resectability and differentiate between benign and malignant lesions.[9] Precise diagnosis is established by biopsy, although very few patients have a histological diagnosis prior to surgery.[4] Despite a lack of standard treatment guidelines, most physicians advocate for early and total surgical tumour excision followed by adjuvant chemotherapy or chemoradiation.[10]

PRIMARY CARDIAC SARCOMA

Primary cardiac sarcomas are malignant tumours that originate from mesenchymal cells and are confined to the heart.[11] They comprise 95% of all malignant primary cardiac tumours and are extremely rare and lethal.[12,13] Some hypothesise that the poor prognosis associated with primary cardiac sarcoma is a function of both local invasion and metastases at presentation.[14] A retrospective analysis of cardiac sarcomas reported a 0.0017% prevalence of cardiac sarcomas at autopsy.[15] Most cardiac sarcomas carry a provisional diagnosis of a benign myxoma until intra-operative observation of invasive neoplasm prompts the suspicion of sarcoma.[16]

Most of the gene alterations associated with sarcomas are chromosomal translocations.[17] Histopathologic analysis allows for directed use of immunohistochemistry and molecular techniques that are instrumental in establishment of diagnosis.[18] Cardiac sarcomas typically have genetic profiles with recurrent alterations in *MDM2, PDGFRA,* or *EGFR,* which have potential as future therapeutic targets.[19] Immunohistochemistry can aid in the classification of cardiac sarcomas and distinguish benign from malignant lesions.[14] However, immunochemistry has a limited value in the setting of evaluation of a cardiac sarcoma due to a frequent lack of morphologically recognisable differentiation, as well as a lack of tissue-specific antigens.[11]

Left-sided cardiac tumours are less infiltrative than right-sided tumours and have a superior overall survival.[20] Primary left-heart sarcomas most frequently originate in the left atrium.[21] Systemic metastases due to cardiac sarcoma are detected in 80% of cases at the time of presentation.[1] Although left-sided cardiac tumours are slower to metastasise, their tendency to grow into the left atrium and obstruct flow often leads to heart failure requiring urgent surgery for alleviation of symptoms.[8] While loco-regional progression is the most common cause of death in patients with cardiac sarcoma, tumour grade is more predictive of the development of metastases.[22]

Due to the rarity of primary cardiac sarcoma, there are no randomised controlled trials or guidelines to determine optimal treatment. Therapy should consider age, performance status, prognosis, clinical symptoms, and tumour characteristics (histology, size, location, and extent of systemic disease), as well as previous thoracic radiotherapy.[23] The majority of primary cardiac sarcomas are high-grade[24] and treatment decisions are primarily guided by anatomic location rather than by tissue type.[25] Poor prognostic factors include tumours with high mitotic index, evidence of tumour necrosis, or invasion of myocardial tissue.[26] In the absence of metastases at diagnosis, surgical resection of a primary cardiac sarcoma is considered the gold standard therapy[11] and is a major determinant of survival.[8] While cardiac function can be preserved in up to 30% of right ventricular surgical resection, left ventricular resections are higher risk.[27] Patients with extensive cardiac metastases, significant intracavitary disease, or uncontrolled disease are poor candidates for surgical resection.[28] Less than half of patients with cardiac sarcoma are able to undergo a complete tumour resection.[29] Obstacles to complete surgical resection include deep invasion of cardiac tissues or tumour location in a site that necessitates intricate reconstruction.[11] As Putnam et al.[30] concluded, "though operative mortality and morbidity may be high, complete resection will yield twice the long-term survival as that of unresectable patients with significant symptom-free survival."

Patients with cardiac sarcoma who receive combination therapy, including surgery, radiation, and chemotherapy, demonstrate a 22.4-month average additional survival benefit in comparison with those treated with surgery, radiation, or chemotherapy alone.[31] A retrospective analysis of surgically resected cardiac sarcomas found

that low-grade tumour histology and survival following initial resection were associated with improved prognosis.[32] While surgical resection is the preferred therapy for cardiac sarcomas, there is a mortality risk of 8.3%.[30] Systemic therapy is frequently used in patients with incomplete surgical resections, recurrent tumours, or in the setting of an especially aggressive neoplasm.[14] Some advocate that chemotherapy has a role even following a complete surgical resection due to the high likelihood of missed microscopic disease.[25] If complete resection is unfeasible, cardiac transplantation may be considered in the absence of metastases. If the tumour is unable to be resected in the setting of metastases, palliative chemotherapy is recommended.[33] Some patients with cardiac sarcoma receive chemotherapy including adriamycin and ifosfamide, or gemcitabine and docetaxel.[8]

While cardiac sarcomas are typically resistant to radiotherapy, sometimes radiotherapy is administered in large high-grade tumours or those with positive or borderline margins following surgical resection.[12] Although radiotherapy has been associated with decreases in local recurrence rates, there is a risk of myocardial damage, including cardiomyopathy and pericarditis, along with minimal or no influence on survival.[14] In the absence of metastases, both orthotopic cardiac transplantation (receipt of a donor's heart) and cardiac autotransplantation (cardiac excision with *ex vivo* tumour resection and re-implantation) are potential therapies in primary cardiac sarcoma.[20,25] Some providers are hesitant to recommend cardiac transplantation given the potential for immunosuppressant therapy to potentiate metastases.[29] In addition, immunosuppression has the potential to induce new neoplastic growth, thereby catalysing the development of another primary malignancy.[14] However, historical data demonstrated that orthotopic heart transplantation has a long-term survival benefit without recurrence, despite use of immunosuppressants.[34]

UNDIFFERENTIATED SPINDLE CELL SARCOMA

Intimal cell sarcoma is a subtype of undifferentiated spindle cell sarcoma, a large and diverse group of tumours composed of elongated cells that arise from the inner layer of the arterial wall. This class of sarcoma is particularly heterogeneous, with variance among their structural composition of nuclei shape, volume of cytoplasm, stromal fibrosis, and myxoid accumulation.[35] Spindle cell sarcomas are usually immunoreactive to vimentin, osteopontin, and MDM2[12,36] with variable positivity for alpha smooth muscle actin, CD117, CD68, p53, and Bcl-2.[16] Complex karyotypes of the *MDM2* gene encompassing the 12q13-14 region are usually present in spindle cell sarcoma.[37] Spindle cell sarcoma lacks a specific and universal histological grading scheme, complicating its diagnosis.[38]

According to an update of the World Health Organization (WHO) 2013 guidelines, spindle cell sarcomas fall into the undifferentiated/unclassified subcategory of sarcomas and account for up to 20% of sarcomas.[39,40] Undifferentiated sarcomas are tumours in which all recognisable tissue differentiation has been excluded;[40] they are further classified by principal type of morphology including round cell, spindle cell, pleomorphic, and epithelioid.[40] Undifferentiated sarcomas are particularly aggressive and high-grade. Remarkably, 80% of patients with spindle cell sarcoma have metastases at the time of diagnosis.[16] Unfortunately, due to its rarity, the management of spindle cell sarcoma is driven mostly by anecdotal data with a lack of evidence-based guidelines.[16] As a result of the rare incidence of spindle cell sarcoma, distinct clinical characteristics, outcomes, organ involvement, and specific prognostic factors remain enigmas.[38] Spindle cell sarcomas typically form polypoid masses that attach to the inner surface of a blood vessel in a manner comparable to a thrombus.[13] Varying expression of cell-cycle markers, specifically a decrease of p27Kip1 expression and an increase of cyclin E expression, may account for the aggressive and invasive nature of spindle cell sarcomas.

PRIMARY CARDIAC SPINDLE CELL SARCOMA

Primary cardiac spindle cell sarcoma is the rarest form of primary cardiac tumour, with very few publicised reports and non-existent treatment guidelines.[41] According to the authors' knowledge, only 15 cases (Figure 1) of primary cardiac spindle cell sarcoma have been reported worldwide.

They are distributed between 12 left atrial tumours,[37,42-52] 1 left ventricle tumour,[53] 1 right ventricle tumour,[16] and 1 pericardial tumour.[54]

The mesenchymal origin of cardiac spindle cell sarcomas explains their predilection to affect large cardiopulmonary blood vessels.[16] Spindle cell sarcomas are typically positive for complex karyotypes encompassing the 12q13-14 region (*MDM2* gene).[37] Immunochemistry stains can detect *MDM2* in cardiac sarcomas that are poorly differentiated and may be confirmed by further studies, including fluorescence *in situ* hybridisation (FISH), quantitative PCR, or array comparative genomic hybridisation analysis.[19] The prognosis of cardiac primary spindle cell sarcomas is poor with a mean survival of 3 months to 1 year.[16] Initial symptoms include blood flow obstruction, embolism, and regional neoplastic infiltration.[16] Presenting symptoms of left atrial primary cardiac spindle cell sarcoma include high fever, mass effect on mitral valve leading to mitral stenosis, and severe congestive heart failure.[55]

Treatment strategies of cardiac spindle cell sarcomas vary and may involve surgery, chemotherapy, and radiation. Regardless of differing surgical techniques, patients with primary cardiac spindle cell sarcoma who underwent surgical resection had more favourable disease-free survival and overall survival in comparison with those who did not receive surgery,[38] which was true regardless of the quality of resection or presence of metastases.[56] Even following a complete surgical resection, patients with primary cardiac spindle cell sarcoma most commonly died from macroscopic local tumour.[43]

LYNCH SYNDROME

Lynch syndrome, also referred to as to as hereditary nonpolyposis colorectal cancer, is an autosomal dominant disorder resulting from germline mutations in the mismatch repair genes (MMR) including *MLH1,*[57] *MSH2*,[58] and *MSH6*.[59,60] Patients with *MLH1* and *MSH2* mutations have a higher risk of malignancy (specifically colorectal cancer) than those with *MSH6* mutations.[61] Interestingly, the rate of ovarian cancer increases markedly at age 40 years for patients with mutations in *MLH1* and *MSH2*.[61] Generally, somatic mutation of an *MMR* gene in a patient with Lynch syndrome results in an increased number of DNA replication errors; these errors are a reflection of the degree of microsatellite instability: i.e., differing lengths of repetitive DNA sequences in tumour-extracted DNA.[62] Defects in mismatch repair genes predispose patients to develop different types of cancer with a guiding symptom of development of usually right-sided colorectal cancer at a young age.[63] Despite the frequency of right-sided colorectal cancer in patients with Lynch syndrome, other neoplasms have also been reported in higher frequencies in patients with Lynch syndrome and include tumours of the stomach, ovaries, pancreas, biliary tract, brain, sebaceous gland adenomas, and keratoacanthomas.[64]

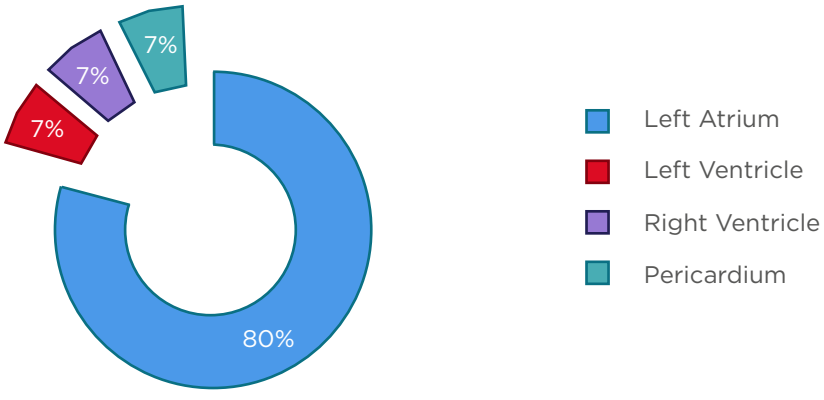

Figure 1: Anatomic distribution of primary cardiac undifferentiated spindle cell sarcoma.

Sarcoma in Lynch Syndrome

Soft tissue sarcomas are neither Lynch syndrome-defining malignancies nor neoplasms that are commonly associated with Lynch syndrome.[65] Diagnosis of a Lynch syndrome-related neoplasm is achieved through microsatellite instability testing of the sarcoma by immunohistochemical staining of MMR proteins.[65] Traditionally, the development of sarcomas is due to alteration of tumour suppressor genes rather than alteration of oncogenes.[66] However, sarcomas may emerge in the setting of Lynch syndrome.[67] While a few sarcomas, including leiomyosarcoma and undifferentiated pleomorphic sarcoma, have been associated with Lynch syndrome, liposarcoma is the most frequent.[67-72]

A prospective study of 658 adults with soft tissue sarcoma revealed that 2.8% of those patients had a genetic syndrome, predominantly Recklinghausen neurofibromatosis and bilateral retinoblastoma.[73] While reports of rhabdomyosarcoma[67] and gastric sarcoma[74] have been identified in patients with Lynch syndrome, no reported cases of cardiac sarcoma or spindle cell sarcoma have been previously reported in patients with either constitutional mismatch-repair deficiency or Lynch syndrome.[70] Documented cases of soft tissue sarcomas in patients with Lynch syndrome families[68,75] may be associated with mutations in *MSH2*, *MSH6*, and *MLH1*.[62,68,69] An analysis of 11 patients with Lynch syndrome and soft tissue sarcomas revealed a germline *MSH2* mutation in over half of the cases. Despite a small number of cases, the authors hypothesise that the risk of soft tissue sarcomas is higher in *MSH2*-deficient patients and families.[71] Notably, patients with a personal or familial history of Lynch syndrome who develop sarcomas demonstrate a predominance of *MSH2* mutations.[70] While some researchers hypothesise the association between soft tissue sarcomas and MMR gene deficiency, particularly *hMSH2*,[71] others question the influence of microsatellite instability and mismatch repair mutations as features of sarcomas.[67] Soft tissue sarcomas in patients with Lynch syndrome have previously been considered coincidental.[69] Further investigation of patients with Lynch syndrome and sarcoma is needed, with precise analysis of mutated MMR genes including *MLH1, MSH2, MSH6, PMS2,* as well as the p53 and retinoblastoma signalling pathways.[68]

CASE REPORT

A 55-year-old woman with a past medical history of Lynch syndrome and ovarian clear cell carcinoma after total abdominal hysterectomy and bilateral salpingo-oophorectomy and six cycles of carboplatin and paclitaxel presented to a local hospital with acute dyspnoea. Family history was remarkable, with a father, paternal uncle, and paternal aunt with colon cancer, as well as a paternal aunt with ovarian cancer. The patient received screening colonoscopies every 3 years that were unremarkable, most recently in November 2017. The patient worked as an administrator and denied tobacco, alcohol, or drug use.

Three months prior to presentation, the patient reported a few-week history of viral respiratory illness that precipitated an acute episode of chest pain, dyspnoea, and near-syncope. The patient was taken to a local hospital where she was found to be in cardiogenic shock (blood pressure 61/43 mmHg) requiring vasopressors. Laboratory testing showed leukocytosis of 18.4 thousand/uL, haemoglobin 13.8 g/dL, alanine aminotransferase 115 U/L, aspartate aminotransferase 124 U/L, and lactic acid 4.4 mmol/L, with the remaining laboratory results within normal limits. Troponin <0.02 ng/mL, brain natriuretic peptide 18 pg/mL, D-dimer 193 ng/mL, and lipase 141 unit/L were all negative. Physical exam was remarkable only for jugular venous distension. CT scan of the chest demonstrated a large pericardial effusion and an echocardiogram revealed a massive circumferential pericardial effusion with early diastolic collapse of the right atrium consistent with early tamponade. A subxiphoid pericardial window placed for cardiac tamponade, fluid cytology was negative, and the patient was discharged with a suspected viral pericarditis.

One month later, screening breast ultrasound and mammogram revealed an irregular hypoechoic mass 9x9x9 mm with two adjacent irregular hypoechoic nodules overlying the right pectoralis muscle. Previous mammograms, most recently 6 years prior, were without any features to suggest malignancy. Over the next few weeks, the patient developed recurrent acute dyspnoea, along with palpitations and chest pain. A physical exam showed a pericardial rub and a right breast mass in the upper-outer quadrant with

associated palpable ipsilateral lymphadenopathy. CT showed a moderate left pleural effusion and a large pericardial effusion with mass effect on the main pulmonary artery, left ventricle, and left pulmonary veins (Figure 2). Transthoracic echocardiogram (TTE) revealed a small and distorted left ventricular cavity, compression of left ventricular wall by a large 10–12 cm loculated semiliquid pericardial mass/effusion, and compression of the main pulmonary artery with peak gradient of 25–30 mmHg. Left anterior mini-thoracotomy revealed an avascular multilobulated mass with left ventricular myocardial invasion and associated tamponade. Additionally, 800 mL of pericardial fluid and 2 L of haemorrhagic left pleural fluid were removed. A biopsy of the left ventricular mass revealed myxoid spindle cell sarcoma of uncertain histogenesis and biopsy of intrapericardial tissue demonstrated benign pericardium with patchy chronic inflammation and fibrinous exudate (Figure 3). Immunostains for calretinin highlighted reactive mesothelial cells in the pericardial tissue and immunostains were negative for Ber-Ep4, MOC-31, MDM2, CDK4, CK, B100, SOX10, CD34, and desmin. NTRK1 immunostain was positive; however, FISH studies did not reveal any genomic abnormality in *NTRK1* or *NTRK3*. Simultaneous biopsy from the right breast revealed poorly differentiated invasive ductal carcinoma that was oestrogen and progesterone receptor negative with an equivocal human epidermal growth factor receptor 2 score (2+) and a Ki67 of 50%. Immunostains showed tumour cells positive for GATA-3, E-cadherin, CK5, and p63 (patchy). Biopsy of axillary lymph nodes was consistent with metastatic carcinoma from tumour in concurrent breast.

The patient was diagnosed with cardiac spindle cell sarcoma (based on sections cT3, cN0, cM0 of the Soft Tissue Sarcoma of the Abdomen and Thoracic Visceral Organs staging form)[76] and was referred for chemotherapy with gemcitabine and docetaxel. Treatment of the cardiac sarcoma was prioritised over the 1.3 cm malignant breast tumour given progressive and debilitating cardiopulmonary symptoms, although the regimen of gemcitabine and docetaxel was selected to treat both. One week following induction chemotherapy, the patient's dyspnoea progressed from exertional-only to present at rest and she presented to the authors' emergency department for further evaluation.

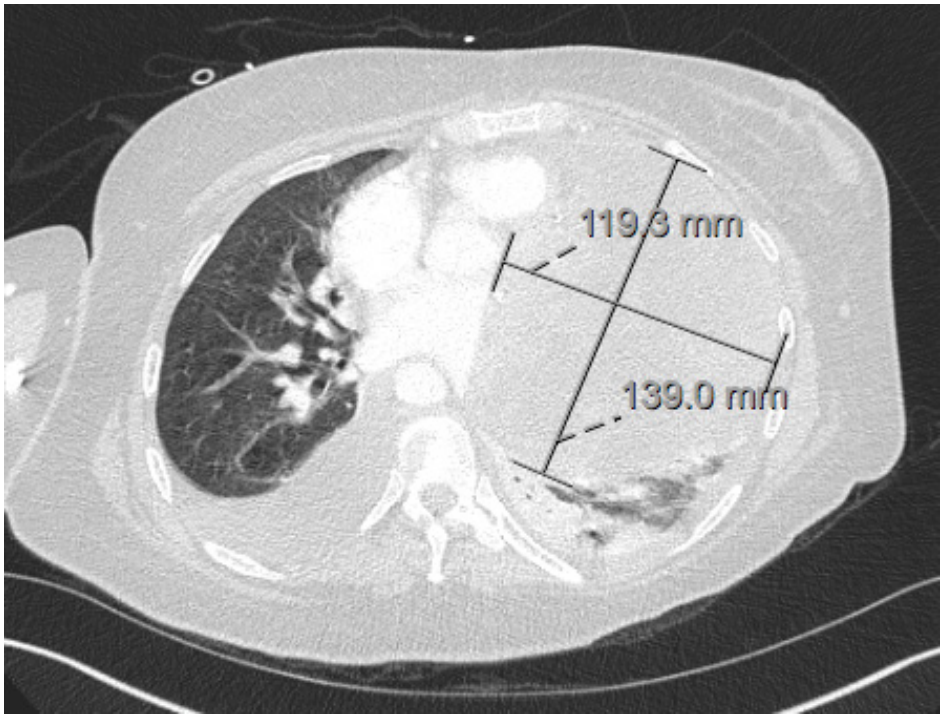

Figure 2: CT showing moderate left pleural effusion and a large pericardial effusion with mass effect on the main pulmonary artery, left ventricle, and left pulmonary veins.

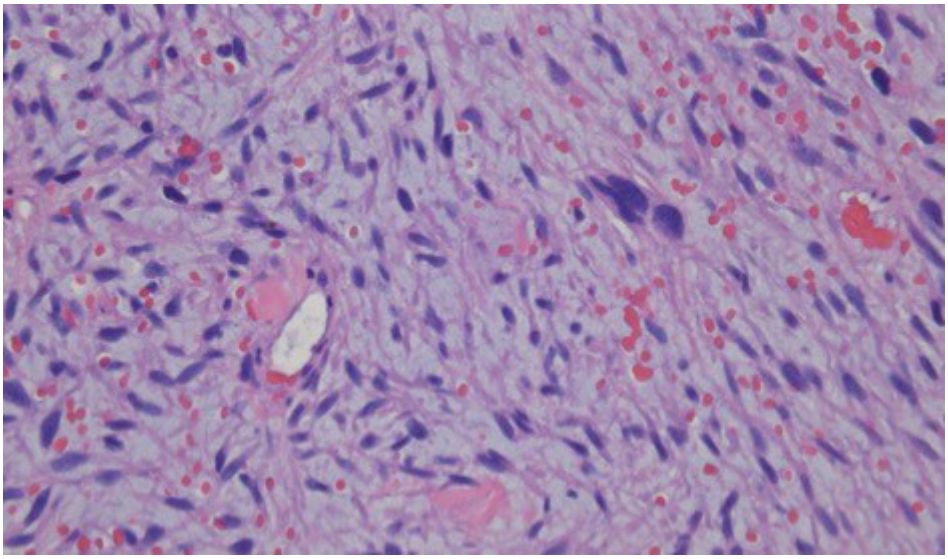

Figure 3: Biopsy of intrapericardial tissue demonstrating benign pericardium with patchy chronic inflammation and fibrinous exudate.

Vital signs on arrival were 114/71 mmHg, heart rate of 70 beats per minute, respiratory rate of 20 breaths per minute, temperature 98 °F, and oxygen saturation was 97%. Laboratory tests were notable for lactic acid 1.5 (mmol/L), troponin <0.011 (ng/mL), and brain natriuretic peptide 2,100 (pg/mL). Chest X-ray with markedly increased large left pleural effusion, increased moderate right pleural effusion, and new superimposed reticulonodular opacity in the right upper lung could. TTE revealed a left ventricular ejection fraction of 60–65%, redemonstration of a large mass extrinsic to the heart in the posterolateral region of the left ventricle, mildly elevated pulmonary artery systolic pressure (31 mmHg), and moderate left ventricular hypertrophy.

Thoracentesis of the left chest yielded 700 cc reddish fluid with reactive mesothelial cells and lymphocytes, without evidence of malignancy. Repeat TTE showed a small left ventricle, left ventricular ejection fraction 60–65%, a small pericardial effusion, an underfilled right ventricle, moderate tricuspid regurgitation, and moderately elevated pulmonary artery systolic pressure (41 mmHg). Given the rapid development and progression of the tumour with severe functional limitations in the setting of the low likelihood of complete surgical resection, the family pursued a palliative approach. The patient died days later, 3 months following initial symptoms and 4 weeks following diagnosis.

CASE DISCUSSION

While there has been only one case, to the authors' knowledge, of a pulmonary artery spindle cell sarcoma in a patient with Lynch syndrome,[77] there are no documented cases of either primary cardiac sarcoma or primary intracardiac spindle cell sarcoma in patients with Lynch syndrome. Moreover, this case represents the first MDM-negative primary cardiac sarcoma ever reported. In addition to the negative MDM immunohistopathologic analysis, this tumour was also negative for vimentin and desmin, which, along with MDM, are immunoreactive in spindle cell sarcoma and serve as principle diagnostic markers.[12,36,45]

To the authors' knowledge, there are only 15 reported cases of primary intracardiac spindle cell sarcoma, 80% of which are tumours of the left atrium. The left ventricular origin of this primary cardiac spindle cell sarcoma differentiates it from most cardiac sarcomas and other primary cardiac spindle cell sarcomas as the second such published case.[53] Some elements of this case presentation were typical of primary cardiac tumours, including haemodynamic dysfunction with induction of cardiac tamponade and initial

presentation with New York Heart Association (NYHA) Class IV symptomatology. However, the absence of metastases at diagnosis is atypical for a left-sided cardiac sarcoma. With >80% of patients with primary cardiac spindle cell sarcoma presenting with metastases at diagnosis,[16] this case defies typical pattern and progression of disease. Left-sided cardiac tumours are typically less invasive and associated with higher rates of overall survival. This cardiac tumour was extremely aggressive with a 3-month survival time following initial symptoms, with sequential echocardiograms illustrating the 4-week development of a mass with compression of both the lateral left ventricular wall and main pulmonary artery. The dimensions of this cardiac mass were particularly large, with a diameter of 12 cm, more than twice the average 5 cm size of a cardiac tumour.[78] Divergent from the tendency of left-sided cardiac tumours to have less invasive growth and improved survival, this case demonstrated rapid and extensive neoplastic infiltration with corresponding severely diminished survival. The simultaneous recognition of two separate primary malignancies not commonly associated with Lynch syndrome, breast carcinoma, and cardiac spindle cell sarcoma, further differentiate this case. The large, invasive mass induced cardiogenic shock and was so intricately intertwined with the left ventricular myocardium that it created an unfeasible surgical resection. Careful selection of chemotherapy agents to treat both the breast cancer and the cardiac sarcoma were considered and the combination of gemcitabine and docetaxel was selected.

CONCLUSION AND FUTURE DIRECTIONS

The authors report the first case of MDM negative primary cardiac sarcoma and the first case of primary intra-cardiac spindle cell sarcoma in a patient with Lynch syndrome. To the authors' knowledge, this is the second case of left ventricular primary cardiac spindle cell sarcoma published to date. The rapidly progressive lethality of spindle cell sarcoma in conjunction with the haemodynamic consequences of cardiac tumours form a hazardous combination with severe implications on cardiac function and patient symptomatology. While sarcoma is not a common cancer in patients with Lynch syndrome, those patients with a family history or personal diagnosis of an MMR deficiency should undergo routine surveillance for Lynch syndrome-related malignancies, including regular colonoscopies. While the rarity of primary cardiac sarcomas precludes the establishment of specific treatment algorithms, further research is needed to optimise therapies and the presence of sarcomas in Lynch syndrome may contain additional links not currently identified. Future studies should emphasise genetic analysis to identify common genes to predict risk of neoplastic development, as well as subsequent gene-specific treatment protocols to treat malignancies.

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Obesity and the Effects of Weight Reduction: A Spotlight on Women with Hormone Receptor-Positive Breast Cancer and Heart Disease

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Abstract

Obesity is a common overlapping risk factor for cancer and cardiovascular disease (CVD), and the long-term consequences of these chronic, interconnected diseases are severe. The importance of CVD in breast cancer (BC) patients and survivors has been well-established, and the potential impact of some BC treatments (such as cardiotoxic effects related to chemotherapy or targeted therapy with the use of doxorubicin or trastuzumab, and radiation therapy, especially in cases of left breast tumours) on the cardiovascular condition necessitates ongoing cardiological surveillance. In addition, the possible reduction of some underlying risk factors is critical to long-term protection of BC patients and survivors.

The concept of obesity dynamically interacting with both BC and CVD is important because it is a modifiable risk factor, and the modern management of obesity deserves emphasis. In particular, for many BC patients and survivors, an effective weight reduction programme integrated with standard anticancer and cardiology therapies can improve patient outcomes.

This review presents the complex relationships between overweight, obesity, CVD, and BC risk and highlights outcomes in post and premenopausal women, focussing on patients with hormone receptor-positive BC. The review provides evidence from epidemiologic, observational, and weight loss intervention trials which have examined the effects of weight reduction programmes on BC outcomes. Such studies have indicated that moderate weight loss, with regular physical exercises or stress reduction, can significantly improve BC outcomes. Future lifestyle intervention trials could support the incorporation of weight loss interventions as an integral element of comprehensive management for BC patients and survivors.

INTRODUCTION

There is a connection between increased body mass, or adipose tissue, and higher risk of developing cancer, particularly in hormone-stimulated neoplastic diseases, such as breast cancer (BC), especially among postmenopausal women.[1,2] In addition, obesity and BC are interrelated via complex hormonal and molecular signalling networks.[3] Obesity is also an

important risk factor for cardiometabolic diseases, including coronary heart disease, arterial hypertension, Type 2 diabetes mellitus (T2DM), and dyslipidaemia.[4] Moreover, the toxicity of some anticancer therapies can adversely impact both oncologic and cardiac outcomes. This can be especially dangerous in women with HER2-positive and hormone receptor (HR)-positive biological subtypes of BC.[4]

At present, numerous patients with malignancies are living longer than ever before because of early detection and the progression of modern oncology therapies.[4] In addition, several new therapies for cardiovascular disease (CVD) are being developed.[4] In this situation, patients who are receiving anticancer treatments and cancer survivors are at risk of subclinical toxicity of different medications, including the negative impact anticancer therapies can have on the cardiovascular (CV) system.[4] Achieving a proper balance between antineoplastic treatment and CV safety is essential in daily practice. While the main efforts of medical practitioners are appropriately targeted on the treatment of BC and CVD, obesity, which is often linked with both these conditions, remains unaddressed. In response to this challenge, this article details recent research and strategies to motivate various members of medical teams to work closely with their BC patients and survivors who are obese. Since obesity is a modifiable risk factor for BC and CVD, accomplishing a moderate, but sustained, weight reduction is a big step forward in terms of improving long-term outcomes for both BC and CVD.

OBESITY AND ITS CONNECTIONS WITH MALIGNANCIES

An imbalance in the endocrine regulation of adipose tissue metabolism often leads to an abnormal fat accumulation and an excess of body mass, causing obesity. Commonly used anthropometric markers of obesity include BMI, waist circumference, and waist-to-hip ratio (measured by waist circumference divided by hip circumference).[5] Individuals can be divided into classes based on their BMI: underweight (<18.5), normal weight (18.5–24.9), overweight (25.0–29.9), or obese (≥30.0), and the waist-to-hip ratio reflects the distribution of the body adiposity by indicating whether more fat is located in the abdomen or the hips.[6] Current research indicates that higher body adiposity is linked to an elevated risk of several cancers, including breast, endometrial, ovarian, oesophageal, gastric, colorectal, liver, renal, pancreatic, thyroid, gallbladder, multiple myeloma, and meningioma.[1] Several studies have shown that an elevated BMI was related to increased risk of BC, especially in postmenopausal women. For instance, obese postmenopausal women had a 20–40% increased risk of developing HR-positive BC, compared with normal weight women.[2] In contrast, overweight and obese premenopausal women had a 20% decreased risk of HR-positive BC compared to normal weight premenopausal women.[2]

COMMON MECHANISMS BY WHICH OBESITY MAY INCREASE THE NEOPLASTIC RISK

Obesity can increase the risk of cancer via several mechanisms that are addressed below. Obese individuals can have chronic, low-level inflammation that can cause damage to DNA, leading to neoplastic lesions.[7] Furthermore, adipose tissue produces an excessive amount of oestrogen that has been related to an elevated risk of hormone-stimulated cancers, such as breast, endometrial, ovarian, and various other malignancies. In addition, obese individuals may be affected by hyperinsulinaemia, insulin resistance, or pre-diabetes, which are disorders that can precede the development of T2DM. Obese and overweight patients often have high blood levels of insulin and insulin-like growth factor-1, which may promote the development of breast, endometrium, colon, kidney, or prostate malignancies.[8] Adipose cells produce adipokines, tissue hormones such as leptin and adiponectin, that can stimulate or inhibit cellular growth or proliferation. Particularly in obese individuals, the level of leptin in the blood increases with growing body mass and fat, and there is a lower level of adiponectin, which has antiproliferative effects, compared to people with a normal body weight. Moreover, adipose cells can have direct or indirect effects on some cell growth regulators, such as a mTOR or adenosine monophosphate (AMP)-activated protein kinase. Examples of additional

biological mechanisms that connect obesity and cancer risk include changes in the immune response, alterations in the NFκB system, and a higher level of oxidative stress.[9]

THE CONNECTIONS BETWEEN BREAST CANCER, OVERWEIGHT, AND OBESITY

The relationship between obesity and BC is complex. BMI has been established as a risk factor for BC, which influences treatment outcomes, predominantly in postmenopausal women.[10] In particular, it has been shown that overweight and obese women with BC have an elevated risk of distant metastases compared with patients who have a BMI within the normal range.[11] Mechanisms connecting obesity and BC include the endocrine and metabolic effects of excessive body mass and fat content, as well as the changes that they cause in molecular signalling pathways and endocrine communication.[3] A meta-analysis has found that each 5 kg increase in adult weight gain was correlated with an 11% increased risk of postmenopausal BC among women who were not using hormone replacement therapy. However, there was no similar relationship between obesity and BC in the case of premenopausal BC.[12] Results of the Nurses' Health Study and Nurses' Health Study II trials have shown that excessive body mass and adiposity in childhood and adolescence were correlated with a 20–50% decrease in the risk of BC across the lifespan, regardless of menopausal status. However, a positive connection has been noted between a short-term weight gain, pre and postmenopausal invasive BC, and HR status, after adjusting for pre and postmenopausal BMI.[13] This correlation was stronger for premenopausal compared with postmenopausal women. Increased risk of BC among postmenopausal women who were lean in adolescence was found in those who had excessive weight gain during adulthood.[14] In addition, in BC survivors, obesity can have an adverse influence on cancer recurrence, complications, and quality of life.[15]

THE RELATIONSHIP BETWEEN OBESITY AND CARDIOVASCULAR DISEASE

Overweight and obesity represent risk factors for cardiometabolic diseases, such as T2DM, arterial hypertension, and dyslipidaemia. Furthermore, the adverse metabolic effects of excess body adiposity accelerate the progression of atheromatic lesions that can, in turn, lead to atherogenic CVD, such as coronary heart disease, stroke, or premature cardiac death.[4] Overweight and obesity, represented by a BMI of ≥25 and ≥30 kg/m^2, respectively, are important modifiable risk factors for CVD.[16] Moreover, excess body mass and central adiposity, which are often associated with physical inactivity, can augment the risk of CVD. In particular, class III obesity (BMI ≥40 kg/m^2) can present a higher CV risk than class I (BMI 30–35 kg/m^2) or class II (BMI 35–40 kg/m^2) obesity among post menopausal women, which is the case across different ethnic populations.[17] Furthermore, there is a link between obesity and thrombosis, including increased expression of the prothrombotic plasminogen activator inhibitor-1 and tissue factor, as well as elevated activity of platelets. Additionally, risk factors for venous thromboembolism among patients with obesity include inflammation, increased thrombin production, decreased fibrinolysis, and platelet hyperactivity.[18] Proinflammatory and prothrombotic factors can aggravate both atherogenic CVD and malignant lesions.

OBESITY: NEW INSIGHTS INTO THE CONTROL OF ENERGY BALANCE

A wide spectrum of genetic, metabolic, behavioural, cultural, and environmental elements contribute to obesity, which has significant medical, psychological, social, and economic consequences.[19] A real breakthrough in obesity research occurred following the discovery that the central nervous system is involved in the accumulation of body fat. The brain plays a key role in the coordination of appetite and body mass.[20] The hypothalamus is the main brain area responsible for the central control of energy intake and expenditure, and the arcuate nucleus in the hypothalamus regulates feeding behaviour and several metabolic pathways.[21] Such a system provides flexible responses to

challenges related to controlling the energy balance (Table 1).[22-24] In these circumstances, sensory, emotional, and psychosocial factors, such as stress, can adversely affect feeding behaviour and body weight, mostly via non-homeostatic mechanisms.[25] On the one hand, the abnormal accumulation of fat tissue can be explained in terms of a disequilibrium between energy intake and expenditure. On the other hand, the mechanisms that determine energy intake and expenditure, in the current environment, are dominated by overwhelming obesogenic factors, such as aggressive food advertisement and the availability of nutrient-depleted, highly processed food, that are beyond simple control.[26,27]

IMPORTANT BENEFITS OF MODERATE WEIGHT LOSS: SMALL STEPS TO IMPROVE LONG-TERM OUTCOMES

Lifestyle modifications in diet, behaviour, and physical activity have been recommended as a primary step in weight reduction.[28]

Table 1: Main signals involved in the homeostatic regulation of appetite and satiety.

Hormone (mediator)	Production (release site)	Physiologic role and practical implications	Reference
Ghrelin	Stomach, small intestines	A hunger hormone. Stimulates food intake, causes increased hunger and food intake.	Howick et al.,[22] 2017
Leptin	White adipose tissue	An anorexigenic satiety hormone that signals nutritional depletion (e.g., fasting or weight loss cause a decrease in leptin levels that in turn triggers changes in food intake, energy expenditure, and neuroendocrine actions, in order to maintain energy balance). Leptin enhances the effects of CCK on inhibition of food intake, but this interaction is disrupted in obesity. Patients with rare mutations in the leptin or leptin receptor gene have early-onset of extreme obesity.	Akieda-Asai et al.,[23] 2014
PYY	L cells in the lower intestine	An anorexigenic satiety hormone that delays gastric emptying, based on the fat content in the meal. Postprandial levels of PYY peak within 2 hours of eating.	De Silva et al.,[24] 2012
CCK	Small intestine, in response to food intake.	Stimulates pancreatic enzymes secretion and intestinal motility, inhibits gastric motility and feeding. CCK can interact with some long-term energy status signals, such as insulin or leptin and may control meal size and regulate body mass.	Akieda-Asai et al.,[23] 2014

Table 1 continued.

Hormone (mediator)	Production (release site)	Physiologic role and practical implications	Reference
GLP-1	Small intestine	Decreases appetite stimulation of GLP-1 receptors, leads to reduced food intake, inhibition of gastric emptying, and increased glucose-stimulated insulin secretion. GLP-1 afferent signals are processed by CNS neurons, that in turn stimulate neuroendocrine, behavioural, and physiologic responses, improving glycaemic control. An antidiabetic medication with a beneficial cardiovascular profile.	De Silva et al.,[24] 2012

CCK: cholecystokinin; CNS: central nervous system; GLP-1: glucagon-like peptide-1; PYY: peptide YY.

Typically, weight loss occurs over 4–6 months, during which time the patients lose 4–10% of their initial body mass. Such a weight reduction is usually followed by a plateau that is followed by weight regain.[29] Since long-term weight loss is still difficult to achieve for many patients, the addition of pharmacotherapy can be considered.[30] The main goal of pharmacotherapy for obesity is to inhibit the biological patterns of weight gain and attenuate the counter-regulatory response of the organism to weight reduction. Improved control of these mechanisms should allow patients to lose weight and sustain body mass reduction.[31] A moderate and sustained body mass reduction, of 5–10% for example, is crucial to improve CV, metabolic, and other disease outcomes.[32] Additionally, weight reduction can decrease proinflammatory markers, such as C-reactive protein, TNF-α, and IL-6.[33] Moreover, weight loss leads to the decrease of insulin secretion and insulin resistance. This, in turn, lowers the risk of CVD and several malignancies.[34] Weight loss can also decrease oestrogen levels, contributing to the reduction of HR-positive BC risk.[35]

INTEGRATION OF WEIGHT REDUCTION AND PHYSICAL ACTIVITY IN BREAST CANCER PATIENTS AND SURVIVORS

The transition from an active anticancer treatment into survivorship is known as the re-entry phase. This can take place during the first year, after completing the adjuvant treatment regimen. The re-entry phase is a convenient stage of the cancer continuum that can be used for education and long-lasting behavioural changes. At that time, the management priorities shift from diagnosis and treatment to long-term survivorship.[36] In fact, the re-entry phase is a prime opportunity for medical teams to actively promote the advantages of healthy behaviours. Studies have shown that losing weight reduces BC risk. In a recent meta-analysis of data from >4 million individuals, weight loss was associated with an 18% relative reduction in BC risk. In addition, exercise, which is often connected with a weight reduction programme, contributed to a 22% relative risk reduction of BC.[37] Furthermore, physical activity may help to decrease some adverse effects of anticancer therapies. For instance, 4 months of resistance and high-intensity training reduced the cancer-related fatigue and symptom burden in BC

patients receiving chemotherapy (CHT).[38] Performing exercises 1 day before a scheduled CHT, such as doxorubicin, improved the patient's mood and reduced the musculoskeletal side effects of the CHT.[39]

THE INVERSE CORRELATION OF PREMENOPAUSAL HORMONE RECEPTOR-POSITIVE BREAST CANCER WITH OVERWEIGHT AND OBESITY: A CHALLENGING TOPIC FOR FURTHER STUDIES

BC has a complex aetiology, in which the body adiposity, commonly assessed via BMI, seems to have opposing effects in postmenopausal and premenopausal patient populations. For example, BC risk is lower premenopause and increases after menopause.[40] Research evidence has shown that the elevated postmenopausal adiposity is positively correlated with BC risk, which is partly due to the increased oestrogen production by excess adipose cells, such as the contribution to HR-positive BC development in the postmenopausal population.[40-42] In addition, increased fat tissue often triggers chronic, low-grade inflammation, which is correlated with a higher risk of BC recurrence.[40] However, according to a recent study, premenopausal women with a higher BMI may have a lower HR-positive BC risk.[43] The results of this study need to be interpreted with caution, due to its limitations.[43] Firstly, this was an observational study; therefore, the findings are not as strong as with a randomised controlled trial (RCT). Secondly, the study used BMI as a measure of the body adiposity, which is not accurate since participants with an identical BMI may have a different amount and distribution of body fat. Thirdly, the weight and height measurements were self-reported, and therefore may have been inaccurate, which would have influenced the findings. Nevertheless, the results of this large, international analysis of data on premenopausal women, aged 18–54 years, have suggested that increased adiposity is associated with a decreased risk of premenopausal BC to a greater degree than previously revealed.[43] In addition, the strongest association of BC risk was found for elevated BMI in early adulthood, at 18–24 years old.[43] At first glance, these findings may seem promising for overweight or obese premenopausal women, in whom an increased adiposity appears to be a protective factor.[43] However, it should be noted that since obesity has several negative effects on health, weight gain should certainly not be recommended for the prevention of BC among premenopausal women.[43] In addition, further studies explaining the exact biological reasons behind the inverse correlation of premenopausal fat with HR-positive BC risk might detect factors contributing to BC, which may have important implications for clinical practice.[43]

EPIDEMIOLOGIC, OBSERVATIONAL, AND WEIGHT LOSS INTERVENTION TRIALS EXPLORING THE EFFECTS ON BREAST CANCER OUTCOMES

Many epidemiologic and observational studies have revealed that obesity, weight gain, and a low level of physical activity have been associated with worse outcomes and survival among women with BC.[40,44] For instance, in a large study, the impact of prognostic factors on BC-specific mortality and non-BC-related mortality, among women with an early stage BC registered in the Surveillance, Epidemiology, and End Results (SEER) programme, was investigated.[45] In general, these women had a good oncologic prognosis. Since most of these patients had died from non-BC-related causes, which are often lifestyle-related, a health-oriented lifestyle together with the comprehensive management of comorbidities should be a priority in such patients.[45] Most women with BC are either overweight or obese at diagnosis, and while they are coping with BC treatments, they are usually unable to lose weight on their own and would benefit from implementing standardised nutrition and exercise programmes.[46] This is in agreement with a recent systematic review that suggested that weight loss is feasible and safe among women who underwent treatment for BC.[47] Similarly, the prospective randomised WINS trial examined the effects of a low-fat diet intervention and demonstrated a mean 3.7% weight loss and decrease in the risk of BC recurrence.[48] Other RCT that were designed to determine whether diet and exercise can improve BC outcomes include the Canadian LISA study[49] and the ENERGY trial,[50] which have shown the benefit of weight loss in BC patients and survivors.

Table 2: Strategies to improve coping with chronic stress in breast cancer patients and survivors.[36]

Useful strategies	Practical examples for patients to consider and implement	Possible advantages
Avoiding unnecessary stressful situations.	Choosing a medical practitioner/team with whom a patient may develop a trusting relationship. Moving closer to the medical centre or finding a healthcare facility closer to home.	Confidence in medical advice. Better compliance with treatment, avoiding miscommunication or discomfort. Decreased transportation stress.
Behavioural modification of the circadian hormonal rhythms, such as cortisol and melatonin release.	Change work schedule to eliminate night-shifts. Stop computer work or watching TV during late evening hours. Stop consumption of alcohol and caffeinated beverages, such as carbonated drinks, tea, and coffee, and tobacco smoking before bedtime.	Improved quality and quantity of sleep. Improved level of functioning. Improved quality of life.
Engaging in supportive health-oriented therapies or pleasant activities.	Join a support group, a properly supervised exercise or walking 'team', a music or art therapy group, or try relaxation therapy. Participate in behavioural therapy, spiritual care, or local community events. Attend workshops for a healthy diet, cooking, gardening, or another safe activity. Develop and maintain positive emotional family and social connections. Cultivate individual hobbies or interests. Adopt a pet. Write a journal. Share valuable experiences directly or via available means of communication, including online, telephone, and local newsletters.	More successful coping with various stressors, related to different stages of diagnostic work-up and therapy of breast cancer. Improved dietary habits, better mood and energy level, improved self-esteem, greater resilience, overcoming loneliness or social stigmatisation due to cancer, and improved quality of life.

Recently, the large-scale, ongoing BWEL RCT has been conducted by the Alliance of Clinical Trials in Oncology. The BWEL trial will examine overweight or obese, premenopausal and postmenopausal patients with BC participating in health education programmes, with or without weight loss intervention, such as loss of 10% of baseline body mass achieved via caloric restriction and increased physical activity, within 1 year of BC diagnosis.[51] The BWEL trial will explore the impact of weight loss on the risk of BC recurrence and mortality.[51]

It is expected that the results of the BWEL trial, which should be available in 2023, will provide convincing data regarding the introduction of a weight loss programme as a standard component of BC management.[51]

THE WEIGHT MANAGEMENT PROGRAMME: JOINT EFFORTS OF MEDICAL TEAM MEMBERS AND PATIENTS

Physicians often have insufficient time to spend during visits with their patients, and thus a close collaboration with dietitians, pharmacists, and nurses is necessary. These practitioners may often devote much more time to the patients

than busy physicians. Moreover, some baseline strategies for coping with chronic stressors aimed at improvement of response to stress, and better control of symptoms, should be adopted by BC patients and survivors (Table 2).[36] Providing such integrated services, together with the educational and behavioural interventions, such as addressing common obstacles to effective co-operation with the medical teams (Table 3),[37,52,53] will improve the psychophysical condition, quality of life, and resilience of many patients with BC and CVD.

Table 3: Possible activities to enhance self-management among obese breast cancer patients and survivors.[37,52,53]

Type of activity	Patient's expectations and views	Medical team's perspectives and tasks	Methodology
Patient education	Explanations of 'why' are more important than providing just 'plain' information about treatment protocols, nutrition, and exercise programmes.	Assess individual patient's education levels; use 'teach-back' techniques.	Brochures, lectures, meetings, and computer-based activities.
Empowering	Good access to medical providers and services; ability to ask questions and to receive instant feedback from the medical team (in the emergency and routine care setting).	Avoid overwhelming patients with medical jargon; provide support during difficult treatment periods.	Scheduling frequent follow-up visits, online contact options, and well-designed patient handouts.
Pharmaceutical care	Ability to ask questions and to receive instant feedback from a pharmacist (e.g., with regard to current medications, their interactions, adverse effects, or interactions with diet).	Conduct regular medication use reviews; provide printed materials to enhance individual teaching.	Online brochures for medication interactions and adverse effects, scheduling consultations.
Precise communication with medical team members	Knowing how to make correct food choices and physical exercises (e.g., nutritional values of meals, and type, intensity, timing, or frequency of exercises), knowing target laboratory test values and medication doses, and knowing basic medical terminology relevant to the patient's case.	Patients need to be told why certain blood tests, imaging studies, or invasive procedures have to be done; why the medications were changed; or the reason behind introducing a new treatment strategy.	Easy access to reliable medical resources online or printed materials to avoid mixed or incorrect messages, scheduling consultations.
Accurate goal setting	Expressing the patient's own goals for breast cancer treatment and weight management.	Asking the patients for their specific goals for the breast cancer treatment and weight management; negotiating mutual, acceptable goals.	Face-to-face visits with medical team members.
Ongoing goal alignment	Encourages patient adherence and commitment to medical care and weight loss and physical exercise programmes.	Facilitates treatment process and builds a professional relationship with patients.	Face-to-face visits, telephone and online contact with medical providers.

CONCLUSION

In summary, BC outcomes are dependent, to a certain degree, on the concurrent CV status and BMI or adipose mass, before, during, and after completing the BC therapy cycle. In addition, the combined efforts of oncology and cardiology specialists, and primary care providers, together with the efficient co-operation with well-educated patients, are crucial to improving BC outcomes and reducing adverse influences of anticancer therapies on the CV condition. In the advent of personalised oncology that applies different targeted therapies to patients with BC, there is a large population of women with excess body weight at risk of possible CV complications. A rapidly growing population of BC survivors, with cardiometabolic comorbidities, represents a big challenge. Under these circumstances, it is imperative to prevent current BC patients from becoming future cardiac patients. In fact, such patients should be the most motivated group since a sustained weight reduction can save their lives. It is expected that further studies will determine whether sustained weight loss can lead to better outcomes among patients with BC. Hopefully, future prospective, large-scale, randomised trials, combining lifestyle interventions with standard medical treatment will support the systematic incorporation of structured weight loss interventions into routine management of patients with BC.

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Anthropometric Parameters and Thyroid Morphology in a Sample of Overweight and Obese Syrian Women

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Abstract

Objective: Obesity and thyroid nodules are very common. Many studies have reported that weight gain is a new risk factor for goiters and thyroid nodules. In this study, the authors aimed to evaluate thyroid morphology in obese women and tried to link thyroid morphology with anthropometric parameters.

Design: A cross-sectional study was conducted between January 2017 and January 2018. Overweight and obese Syrian females, aged >35 years, without past or recent thyroid complaints, and living in the southern region of Syria were included in the study. Weight, height, and hip and waist circumference were measured to calculate anthropometric parameters. Thyroid volume and prevalence of thyroid nodules were compared among BMI groups as defined by World Health Organization classifications. Body Surface Area (BSA), waist circumference (WC), waist:hip ratio, and waist-to-height ratio (WtHR) were compared between nodule groups.

Results: A total of 140 overweight and obese females, with a mean age of aged 53±7 years were studied. Mean thyroid volume was 12.4±7.4 mL, with the highest volume identified in the Obesity Class III group (14.9±10.9 mL). Significant positive correlations were found between thyroid volume and weight, BMI, WC, BSA, and WtHR ($p<0.05$). Thyroid nodule prevalence was 84.3%, and 22.0% of the nodules were fine needle aspiration indicated. Weight and BSA were positively correlated with thyroid nodules ($p<0.05$), while BMI was not.

Conclusion: Thyroid volume was positively correlated with increased weight, BMI, WC, BSA, and WtHR in Syrian females. Positive correlations were found between weight, BSA, and thyroid nodules after age and thyroid stimulating hormone were excluded.

INTRODUCTION

A goiter is a nodular or diffuse enlargement of the thyroid gland. It is defined as an adaptive response of the thyroid follicular cells to any factor that impairs thyroid hormone synthesis.[1,2] Genetics, iodine deficiency, and thyroid stimulating hormone (TSH) are the most important factors that contribute to elevated thyroid volume and nodules formation,[2-4] with women and the elderly more likely to have thyroid nodules.[5-7] Some studies have suggested other risk factors, such as smoking, obesity, and insulin resistance, contribute to nodule formation.[3,8-10] Recently, the association between metabolic obesity and thyroid morphology has received attention. While some studies demonstrated a significant positive correlation between obesity and altered thyroid morphology,[6,11-13] others did not.[14-16] BMI, body surface area (BSA), waist circumference (WC), waist:hip ratio (WHR), and waist-to-height ratio (WtHR) are clinical parameters of body fat and visceral obesity.[17,18]

In this study, the authors evaluate thyroid gland morphology, via ultrasonography, in a sample of overweight and obese females, and examine its relation to BMI and other anthropometric parameters in a mild iodine deficiency area.[19]

METHODS

Population Features

A cross-sectional study was conducted between January 2017 and January 2018. Participants were females aged >35 years and had lived in southern Syria (an area of mild iodine deficiency)[20] for the last 10 years. The participants were selected from outpatient clinics, patients in the hospital wards, and their relatives at Al-Mouwasat University Hospital, Damascus, Syria. Subjects with previous or recent thyroid complaints were excluded. All individuals who gave informed consent were interviewed using a questionnaire that collected information regarding patient age, demographic characteristics, medical history, family history of thyroid disease, smoking, and dietary iodised salt.

Anthropometric Parameters

Weight (with light clothes) and barefoot height were measured using the Seca Scale Model 713 device (Boian Surgical, Padstow, Australia). Waist circumference (the midpoint between the top of the iliac crest and the lower margin of the last palpable rib) and hip circumference (around the widest portion of the buttocks)[17] were measured by the same physician with a flexible tape. BMI, BSA, WHR, and WtHR were calculated as follows:

- BMI = weight (kg)/height (m)^2.
- BSA (Dubois formula) = 0.007184×(height [m]$^{0.725}$)×(weight [kg]$^{0.425}$).[21]
- WHR = waist circumference (cm)/hip circumference (cm).[17]
- WtHR = waist circumference (cm)/height (cm).[18]

Depending on BMI, participants were classified into four groups (overweight [BMI: 25.0–29.9 kg/m^2], Obesity Class I [BMI: 30.0–34.9 kg/m^2], Obesity Class II [BMI: 35.0–39.9 kg/m^2], and Obesity Class III [BMI: ≥40 kg/m^2]) according to the World Health Organization classification.[22] All subjects with BMI <25 kg/m^2 were excluded.

Thyroid Morphology

Thyroid ultrasound, using a L14-6N (6-14 Mhz) Mindray (Providian Medical Equipment, Highland Heights, Ohio, USA) ultrasound device, was performed with the patients in the supine position. Total thyroid volume was determined by the addition of the volumes of both lobes. The lobe volume was calculated using the formula: (length x width x thickness x π/6).[23] Isthmus volume was ignored unless it had nodules, whereupon their volumes were calculated and added to the total volume. A goiter was defined as thyroid volume ≥18 mL in females.[24,25] Each detected nodule (≥2 mm) in the gland was evaluated for fine needle aspiration (FNA) indications according to the American Thyroid Association (ATA) criteria.[26]

Serum TSH was measured in the central laboratory of the hospital, using either Icroma™ (Boditech Med Inc., Chungcheon-si, South Korea), IMMULITE® (Siemens Healthineers, Erlangen, Germany), or Liaison® XL (DiaSorin, Saluggia, Italy) kits, with a 0.3–5.0 µIU/mL reference range.

STATISTICAL ANALYSES

Data analysis was performed using the SPSS software version 23.0 (IBM, Armonk, New York, USA), where p values <0.05 were considered statistically significant. Competitive data were compared among different groups based on variable distribution using the Kolmogorov–Smirnov test. T test and analysis of variance (ANOVA) were used for normal distribution, while Mann–Whitney and Kruskal–Wallis analyses were used as nonparametric tests. The chi square test was used for nominal data and Spearman's rho for correlation.

RESULTS

General Characteristics

A total of 140 overweight or obese females were studied. Their mean age was 53±7 years (Table 1). Mean BMI was 34.2±5.1 kg/m^2 (24.2% overweight, 32.9% Obesity Class I, 27.9% Obesity Class II, and 15.0% Obesity Class III). In total, 87.9% of subjects had a WC of >88 cm and 85.9% had a WHR of ≥0.85.

Thyroid Volume and Anthropometric Parameters

Goiter (thyroid volume >18 mL) prevalence was 10.7%. Significant differences were found in thyroid volume among BMI groups (p<0.05). The Obesity Class III group had the highest thyroid volume (14.9±10.9 mL) (Table 2). Spearman's rho showed a statistically significant positive correlation between thyroid volume and BMI before and after excluding age and TSH as confounding factors in partial correlation (r=0.24; p=0.004 and r=0.23; p=0.008, respectively). Thyroid volume was also positively correlated with weight, WC, BSA, and WtHR (p<0.05) (Table 3).

Thyroid Nodules and Anthropometric Parameters

Thyroid nodules were detected in 84.6% of the participants, 22% of which had FNA indication. The patients were divided into three groups: no nodules (15.7%), nodules without FNA indication (65.7%), and nodules with FNA indication (18.6%) (Table 4). Weight, WC, and BSA were higher in nodule groups with significant difference (p<0.05). There were significant positive correlations between weight, BSA, and thyroid nodules after age and TSH were excluded (r=0.17, p=0.049).

Table 1: Characteristics of the 140 female patients included in the study.

Parameters	Mean±standard deviation
Age (years)	53±7
Weight (kg)	80±12
BMI (kg/m^2)	34.2±5.1
BSA (m^2)	1.75±0.13
Waist circumference (cm)	101±10
WHR	0.92±0.08
WtHR	0.66±0.07
TV (mL)	12.4±7.4
TSH (µu/mL)	1.7±1.1
Thyroid ultrasound	**Percentage**
Nodular thyroid	84.3%
Nodules with FNA indication	18.6%
Goiter (TV>18 mL)	10.7%

BSA: body surface area; TSH: thyroid stimulating hormone; TV: thyroid volume; WHR: waist:hip ratio; WtHR: waist-to-height ratio.

Table 2: Comparison of thyroid volume and thyroid stimulating hormone among BMI groups.

	Percentage	TV (mL)*	p value	TSH (µu/mL)*	p	Age (years)*	p
Overweight	24.2%	9.9±4.4	0.02†	1.5±0.9	0.2	54±7	0.5
Obesity I	32.9%	12.2±6.3		1.7±1.1		53±6	
Obesity II	27.9%	13.3±8.0		1.9±1.1		53±7	
Obesity III	15.0%	14.9±10.9		1.8±1.2		52±6	

*Mean±standard deviation

†Statistically significant

TSH: thyroid stimulating hormone; TV: thyroid volume.

Table 3: Correlations between thyroid volume and anthropometric parameters.

	Spearman's rho		Partial correlation after excluding age and TSH	
Correlation between thyroid volume and:	**r**	**p value**	**r**	**p value**
Weight	0.26	0.001*	0.26	0.003*
BMI	0.24	0.004*	0.23	0.008*
BSA	0.28	0.001*	0.24	0.005*
WC	0.22	0.009*	0.22	0.010*
WHR	0.06	0.440	0.12	0.170
WtHR	0.18	0.020*	0.20	0.020*

*Statistically significant

BSA: body surface area; TSH: thyroid stimulating hormone; TV: thyroid volume; WC: waist circumference; WHR: waist:hip ratio; WtHR: waist-to-height ratio.

DISCUSSION

Obesity is a complex, multifactorial, chronic disease. Its prevalence has been increasing dramatically worldwide, almost tripling between 1975 and 2016.[27] Obesity is considered a major risk factor for diabetes, hypertension, and cardiovascular diseases. Recently, many studies have tried to find other comorbidities associated with obesity. Thyroid disorders are the most common endocrine diseases. High prevalence of goiter and incidental thyroid nodules has led researchers to investigate their relationship with obesity.

In this study, the authors evaluated thyroid gland morphology in a sample of middle-aged, obese females with no thyroid complaints. The Obesity Class III group had the highest goiter prevalence and the largest thyroid volume. Positive correlations were found between thyroid volume and other anthropometric parameters (weight, BSA, WtHR, and WC), but not WHR.

These results were aligned with those of many other studies.[28-32] Dauksiene et al.[29] showed that BMI was an independent predictor of goiter development. Eray et al.[32] showed that thyroid volume was positively correlated with BMI, with an important reduction in weight loss after 6 months. Turcios et al.[28] found that BMI and BSA were positively linked to thyroid volume but WHR was not. WtHR, as a metabolic parameter, has not been studied with thyroid volume before. The positive results presented here indicate its importance as an independent predictor for thyroid volume.

Table 4: Comparison of anthropometric parameters among nodule groups.

	NO nodules*	Nodules without FNA indication*	Nodules with FNA indication*	p value
Age (years)	53±7	53±6	54±8	0.710
Weight (kg)	76±10	79±12	85±12	0.02†
BMI (kg/m²)	33.0±5.3	34.0±5.1	35.8±4.9	0.140
BSA (m²)	1.71±0.10	1.75±0.13	1.83±0.13	0.005†
WC (cm)	98±10	101±10	106±10	0.040†
WHR	0.90±0.07	0.92±0.08	0.95±0.10	0.170
WtHR	0.65±0.08	0.66±0.07	0.68±0.06	0.230

*Mean±standard deviation

†Statistically significant

BSA: body surface area; FNA: fine needle aspiration; TSH: thyroid stimulating hormone; TV: thyroid volume; WC: waist circumference; WHR: waist:hip ratio; WtHR: waist-to-height ratio.

Regarding thyroid nodules, a high prevalence of thyroid nodules (84.6%) was documented in the mildly iodine deficient southern region of Syria; this was higher than the mean prevalence in other studies, including 44.9%, 34.2%, and 35.4% in Yemen,[33] South Korea[6] and China,[11] respectively. It is hypothesised that the high prevalence maybe due to the age group and female sex. Values of weight, BSA, and WC were the highest in the FNA-indicated nodules group in a statically significant pattern, while BMI and WHR were insignificant. Panagiotou et al.[16] reported that WC was the only anthropometric parametric value that was significantly higher in the nodules groups compared to the no nodules group. Song et al.[13] found a positive significant correlation between thyroid nodules and BMI and BSA, which became insignificant with BMI after being modulated by age and TSH as confounding factors. In a Korean survey, on apparently healthy people, Moon et al.[6] documented that WC and BMI were highest in the thyroid nodules group.

The mechanism of the association between obesity and thyroid morphology is not entirely understood. Obesity is characterised by many complicated mechanisms, of which insulin resistance is the most important.[34] Hyperinsulinaemia, in the case of insulin resistance, can up-regulate hepatic growth hormone receptors and suppress insulin-like growth factors binding proteins, leading to increased bioactive insulin-like growth factor 1 (IGF1) levels.[35] Insulin and IGF1 act via their highly homologous receptors,[36,37] which are expressed in thyrocytes by TSH.[38] They synergise with TSH, enhancing proliferation and differentiation of thyroid cells,[38] and having anti-apoptotic effects (Igf1).[39] This hypothesis is supported by the findings of Anil et al.,[40] who demonstrated that metformin (an insulin sensitiser agent) therapy significantly decreased thyroid volume in subjects with insulin resistance. Insulin resistance may explain the goitrogenic effects of obesity, but more studies are still needed.

LIMITATIONS

In this study, the authors faced many limitations. The focus was placed on thyroid dimensions to calculate the volume, but this did not evaluate thyroid echogenicity. The study did not include grey-scale analysis[41] and could not detect the possible accumulation of lipids in the thyroid in cases of high BMI. In addition, there was a lack of description of autoimmune thyroiditis echographic features which may be present in patients with obesis.[41]

CONCLUSION

This study indicates an association between obesity and thyroid morphology in middle-aged

Syrian females. High prevalence of goiter and thyroid nodules in obese females should highlight the importance of evaluating the thyroid gland using thyroid ultrasound as a screening test. Early detection of thyroid nodules will result in a better prognosis.

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After Conventional Wisdom Has Failed, What Drives Wound Healing?

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Abstract

Between 2006 and 2015, the U.S. Food and Drug Administration's (FDA) overall likelihood of approval (LOA) from Phase I clinical trials for all therapeutic candidates was 9.6%, with the highest LOA in haematology (26.1%) and the lowest in oncology (5.1%). Two critical features attributed to the success of advancing trials were i) targeting driver genes responsible for disease, and ii) use of human disease-relevant animal models during preclinical studies. For decades, conventional wisdom has been that growth factors are the drivers of wound healing, but few have either advanced to clinical applications or proven effective. The purpose of this paper is to explore heat shock protein 90-alpha (Hsp90α)'s role as a potential driver of wound healing and as a possible future therapeutic entity through a review of recent literature, including studies with human disease-relevant animal models. Of the approximately 7,000 gene products generated by a given mammalian cell type, the Hsp90 family of proteins (Hsp90α and Hsp90β) accounts for 2–3% of them. Hsp90β fulfils the role of an intracellular chaperone, but Hsp90α's intracellular function is surprisingly dispensable. Instead, the abundancy of Hsp90α appears to have been prepared for extracellular purposes. When secreted via exosomes by cells under environmental stress, such as injury, Hsp90α protects cells from hypoxia-induced cell death, reduces local inflammation, and subsequently promotes cell migration to repair the injured tissue. Unlike conventional growth factors, secreted Hsp90α stimulates all major cell types involved in wound healing equally, resists microenvironmental inhibitors like TGFβ and hyperglycaemia, and is highly stable. Inhibition of exosome-mediated Hsp90α secretion, neutralisation of Hsp90α's ATPase-independent extracellular functions, or interruption of Hsp90α-LRP-1 signalling blocks wound closure *in vivo*. Topical application of Hsp90α's therapeutic entity, F-5 (a 115-amino acid peptide), has shown great promise for healing acute burn and diabetic wounds in mice and pigs.

INTRODUCTION

According to the Wound Healing Society (WHS), approximately 15% of older adults in the USA suffer from chronic wounds, such as venous stasis ulcers, pressure ulcers (bedsores), and diabetic (neuropathic) foot ulcers, as well as other non-healing wounds caused by bacterial infections, ischaemia, and surgical procedures.[1,2] Every year, 2–3 million Americans are diagnosed with various types of chronic wounds. Using diabetic foot ulcers (DFU) as a well-studied example, the impact of the disease burden of chronic wounds becomes staggeringly clear. Worldwide, 10–25 million diabetics develop DFU annually. In the USA, each of approximately 28 million diabetics has a 25% lifetime risk of developing a DFU.[3-5] DFU are the leading cause of hospital admissions among diabetics and are associated with nearly 70% of all lower leg amputations, a morbidity that more commonly afflicts low-income individuals, members of minority groups, and those without health insurance.[6] Unfortunately, the care currently available, which consists of antibiotics, routine debridement, negative pressure, hyperbaric oxygen, bioengineered skin equivalent grafting, and growth factor therapy, has shown either modest or little efficacy and at a very high cost. This paper explores the potential and reasoning behind the use of a previously unidentified therapeutic candidate, extracellular heat shock protein 90-alpha (Hsp90α), through a focussed review of recent literature.

THE WOUND CLOSURE PHASE OF WOUND HEALING

Wound healing is achieved through several overlapping processes: blood clotting (haemostasis), inflammation (immune system response), tissue growth (proliferation), and tissue remodelling (maturation). The first three processes complete within a few weeks after wounding and are collectively known as 'the wound closure phase,' the most critical and potentially life-threatening phase of wound healing. The remodelling phase following wound closure lasts at least a year and is difficult to follow in animal model experiments. Thus, the development of wound healing therapeutics has primarily targeted wound closure events; however, despite decades of effort, researchers have failed to identify the true driver genes responsible for promoting wound closure. The conventional target, a large serum polypeptide family known as growth factors, has led to minimal therapeutic innovation.[7-9]

Since the discovery of the first growth factor in the 1970s, locally released growth factors in injured tissues were believed to constitute the main driving force behind wound closure.[10,11] Under this assumption, growth factors are responsible for inducing wound re-epithelialisation via promotion of the lateral migration of epidermal keratinocytes, as well as by subsequently acting as chemoattractants to induce the inward migration of dermal fibroblasts (which remodel damaged tissues) and microvascular endothelial cells (which revascularise the neodermis). The first growth factor, epidermal growth factor, entered clinical trials for treating partial thickness skin graft wounds in 1989.[12] Subsequently, more than a dozen other growth factor trials were conducted, including i) epidermal growth factor treatment for traumatic corneal epithelial defects,[13] chronically perforated tympanic membranes,[14] and advanced DFU;[15,16] ii) basic fibroblast growth factor (FGF) treatment for partial-thickness paediatric burn wounds,[17] second-degree burns,[18] and DFU;[19] iii) acidic FGF treatment for partial thickness burns and skin graft donor sites;[20] iv) granulocyte and macrophage colony-stimulating factor plus basic FGF treatment for pressure ulcers;[21] and v) platelet-derived growth factor-BB (PDGF-BB) treatment for chronic pressure and diabetic ulcers.[22-27] Most of these double-blinded trials reported promising clinical efficacy; however, only recombinant human PDGF-BB received the U.S. Food and Drug Administration's (FDA) approval for treatment of DFU (becaplermin gel).[28] Unfortunately, following its approval in 1997, multicentre, randomised, and parallel trials showed that becaplermin gel improved wound closure rates by 15% at best (50% improvement with treatment versus 36% with placebo).[29-31] These outcomes were not considered cost-effective for clinical practice.[23,24] Moreover, in 2008, the FDA added a black box warning to becaplermin gel regarding the increased risk of cancer mortality in patients requiring extensive treatments (≥3 tubes). PDGF-

BB is one of the most potent natural mitogens identified and its dosage in becaplermin gel for clinical use is >1,000-fold the physiological PDGF-BB levels in human circulation.[28] No other growth factors have since advanced to FDA approval.

WHAT WENT WRONG WITH THE CONVENTIONAL WISDOM?

To investigate this important question, the authors evaluated the biological activity of the FDA-approved PDGF-BB, becarplermin gel, as an example and found several possible explanations. Firstly, it is known that most growth factors engage distinct cell types selectively. Between keratinocytes, dermal fibroblasts, and microvascular endothelial cells (three cell types critical to successful wound healing), PDGF-BB can only engage PDGF receptor-positive dermal fibroblasts; neither keratinocytes nor endothelial cells express PDGF receptors.[32] Secondly, members of the TGFβ family of cytokines present in the wound bed are known inhibitors of primary cell migration and proliferation and the authors have reported that TGFβ3 specifically nullifies the effectiveness of growth factors in the wound bed.[33] Please note that this finding does not downplay the importance of TGFβ in extracellular matrix expression and deposition during the later phase of wound remodelling. Thirdly, pathological conditions, such as hypoxia (prevalent in wounds due to vascular disruption) and hyperglycaemia, further compromise the effectiveness of growth factors.[32,34] In fact, vascular injury and obstruction in wounded tissue cause ischaemia, impairing growth factor delivery and creating a hypoxic microenvironment. These three hurdles, as illustrated in Figure 1, reduce the likelihood that growth factors are drivers of early-phase wound closure and lead the authors to speculate that the true driver lies elsewhere.[35]

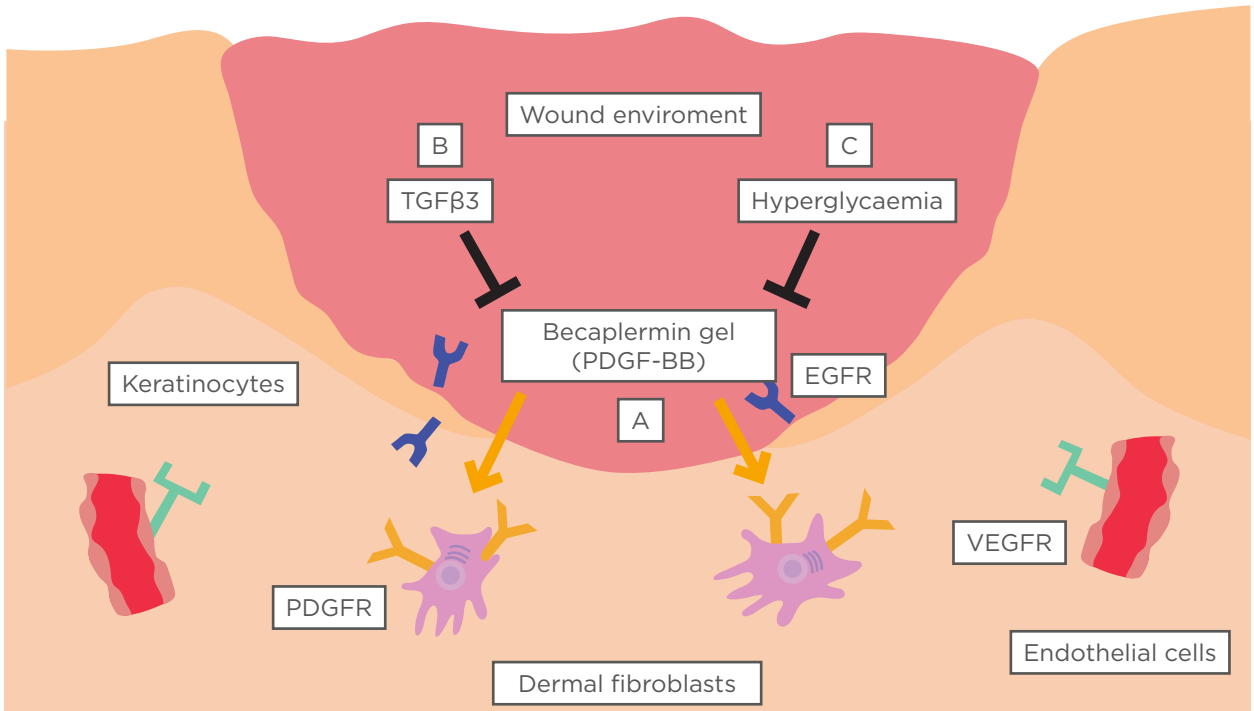

Figure 1: A conventional growth factor cannot be the driver for wound closure.

The three biological hurdles that prevent conventional growth factors from being effective during the early phase of wound closure are: A) selective targeting of only one cell type by any given growth factor; B) complete inhibition of growth factor functionality by concurrent presence of TGFβ in the wound bed; and C) brief, 2–10 minute half-lives within the unfavourable pathological microenvironment of the wound bed.

EGFR: epidermal growth factor receptor; PDGFR: platelet-derived growth factor receptor; PDGF-BB: platelet-derived growth factor subunit B (homodimer); VEGFR: vascular endothelial growth factor receptor.

DISCOVERY OF A NEW POTENTIAL DRIVER FOR WOUND CLOSURE: KERATINOCYTE-SECRETED HEAT SHOCK PROTEIN-90ALPHA

The authors postulated that the driver molecule probably originated from factors secreted by keratinocytes and/or fibroblasts under stress around the wound bed and, more importantly, that this factor must be able to overcome the three hurdles that make growth factors unlikely candidates. Using a sensitive cell motility assay as the standard of detection, protein purification from conditioned media of hypoxia-stressed primary human keratinocytes and dermal fibroblasts led to identification of the secreted form of Hsp90α.[36,37] It was demonstrated that secreted Hsp90α i) acts as a pro-motility factor for all three skin cell types involved in wound closure, ii) is able to override TGFβ inhibition, and iii) remains fully functional even in the hypoxic and hyperglycaemic diabetic wound environment.[38-40] Topical application of human recombinant Hsp90α protein accelerated full-thickness excision, burn, and diabetic skin wound closure by an average of 60% compared to the 15% reduction in closure time achieved by PDGF-BB (becaplermin gel, 0.01%) in mouse and pig models.[32,34,38]

A unique mouse model was generated in which the intracellular function of Hsp90α was selectively silenced by C-terminal deletion, but the extracellular function of Hsp90α, as well as constitutive expression of Hsp90β, was spared. These mice are phenotypically indistinguishable from wild-type (WT) counterparts.[39] This mouse model allowed the authors to specifically assess the importance of Hsp90α's extracellular non-chaperone function and determine whether it is essential for normal wound closure. The authors found that the chaperone-defective Hsp90α–Δ mutant mice experienced wound closure rates similar to Hsp90α-WT mice, suggesting that the secreted Hsp90α–Δ deletion mutant protein was fully functional in the promotion of wound closure. To prove this hypothesis, the authors cloned the mouse complementary DNA encoding Hsp90α–Δ and Hsp90α-WT and generated recombinant proteins. Topical application of Hsp90α–Δ mutant protein promoted wound closure as effectively as full-length Hsp90α-WT protein. Moreover, using 1G6-D7, an anti-Hsp90α monoclonal antibody developed in the authors' laboratory,[40] the authors showed that selective inhibition of extracellular Hsp90α–Δ protein function delayed wound closure in these mice.[41] An earlier study by Song and Luo,[42] who used a nude mouse model and neutralising antibodies against Hsp90, reported similar findings. Taken together, these studies provide direct evidence that extracellular Hsp90α acts as a potential driver for normal wound closure.

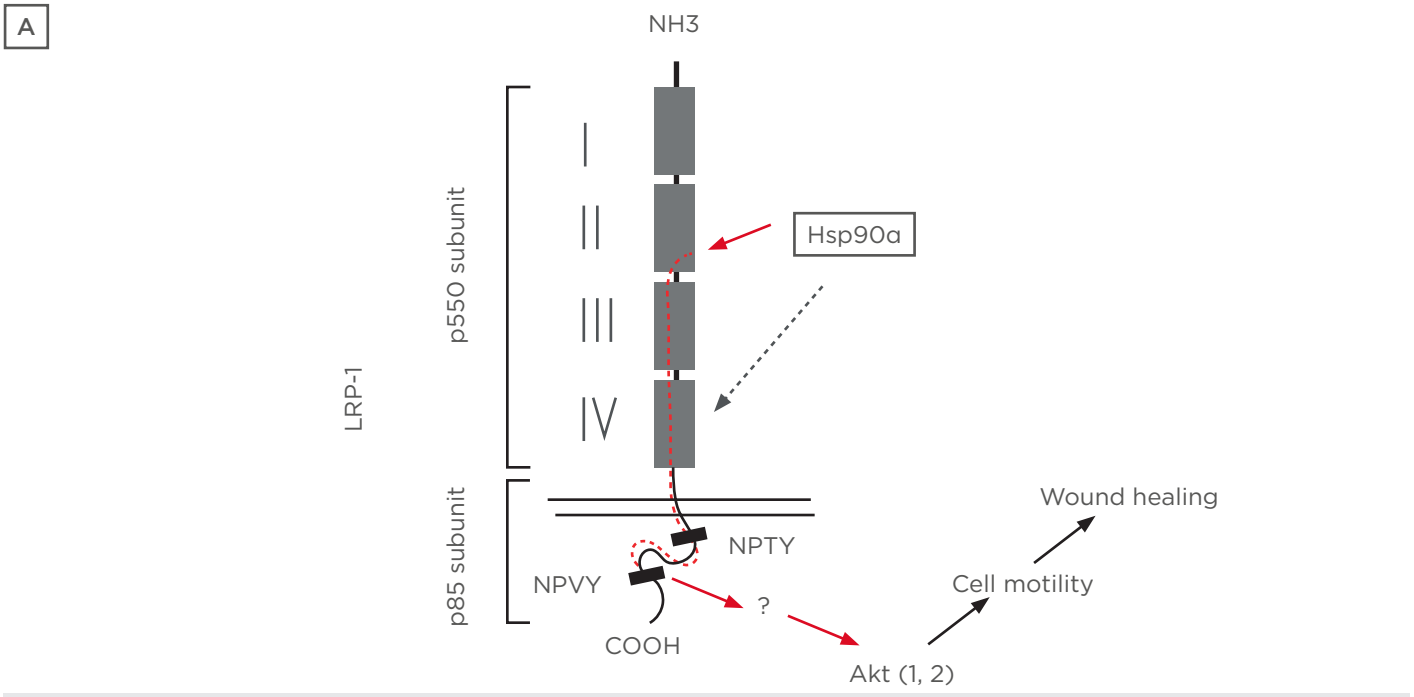

Figure 2: A fundamental and previously less recognised distinction between Hsp90α and Hsp90β.

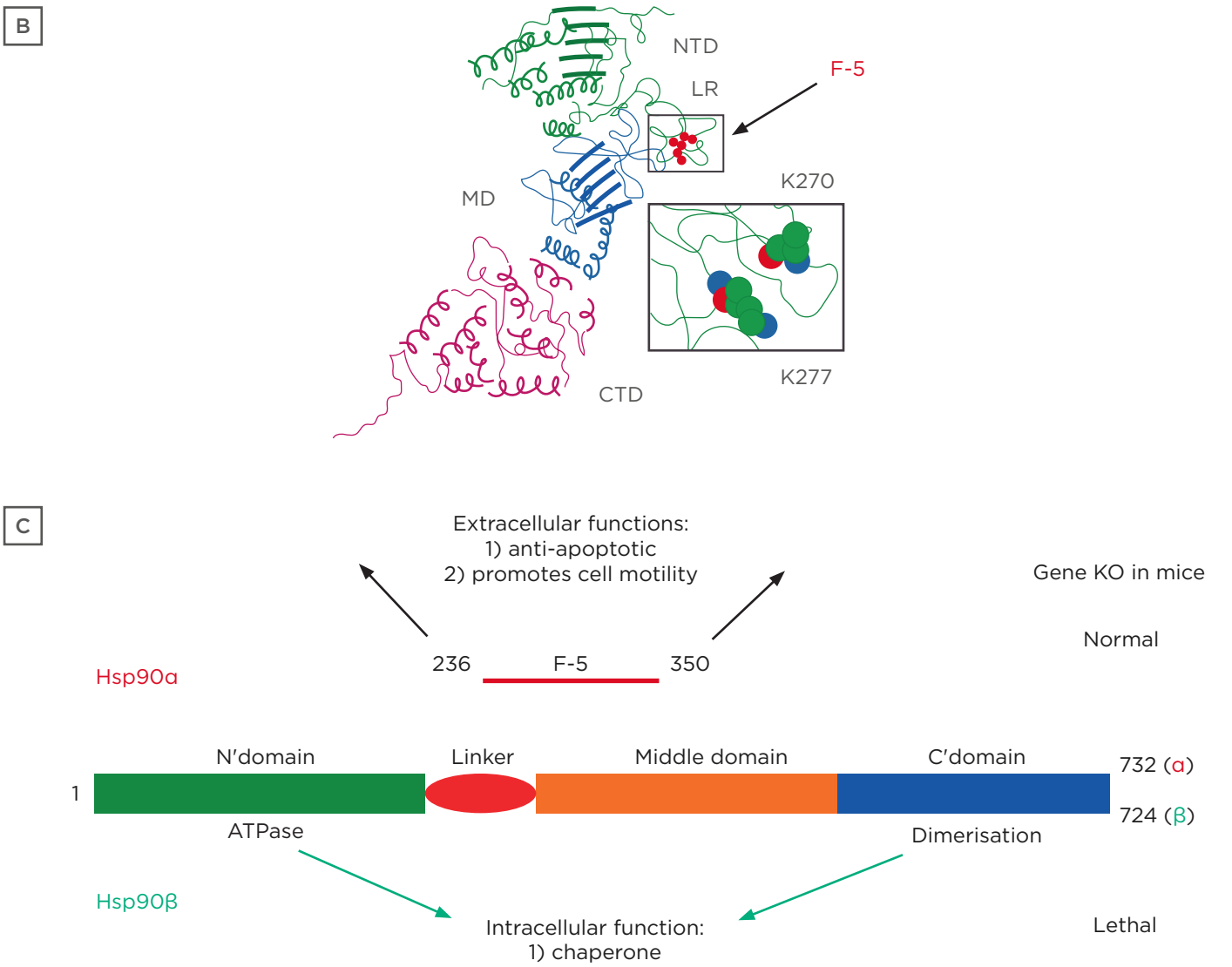

Figure 2 continued.

A) Secreted Hsp90α binds to subdomain II in the extracellular portion of the LRP-1 and activates the LRP-1 receptor.

B) A three-dimensional visualisation of an extracellular F-5 fragment.

C) Under physiological conditions, Hsp90β is the critical intracellular chaperone for homeostasis, whereas Hsp90α is dispensable. Under pathological conditions, such as tissue injury, Hsp90α is secreted via exosomes to the extracellular environment. Once outside of cells, Hsp90α first prevents cells around the wound bed from undergoing hypoxia-induced apoptosis and, thereafter, promotes inward migration of keratinocytes, fibroblasts, and microvascular endothelial cells to close the wound.

Hsp90: heat shock protein-90; KO: knockout; LRP-1: low-density lipoprotein receptor-related protein-1.

SECRETED HEAT SHOCK PROTEIN-90ALPHA IS NOT A CHAPERONE

Mechanistically, as illustrated in Figure 2A, secreted Hsp90α binds to subdomain II in the extracellular portion of the low-density lipoprotein receptor-related protein-1 (LRP-1) and activates the LRP-1 receptor. The NPVY motif in the cytoplasmic tail of LRP-1 connects extracellular Hsp90α signalling to serine-473, but not threonine-308, phosphorylation in Akt kinases. Akt1 and Akt2 work in concert to mediate extracellular Hsp90α's cross-membrane signalling to promote cell survival, cell motility, and wound closure.[43] The Hsp90α-WT protein, Hsp90α-E47D mutant, and Hsp90α-E47A and Hsp90α-D93N mutants have 100%, 50%, and undetectable ATPase activity, respectively.[44]

These constructs allowed Cheng et al.[37] to test whether the chaperone-dependent ATPase is still required for the extracellular function of secreted Hsp90α. Using recombinant proteins encoded by these Hsp90α complementary DNA and cell motility assays, they reported that all the ATPase mutant proteins retained similar degrees of pro-motility activity compared to Hsp90α-WT. Moreover, these authors narrowed the functional entity of secreted Hsp90α to a 115-amino acid fragment, called F-5 (aa-236–aa-350), inside the linker region and middle domain. The extracellular F-5 fragment is visualised in three dimensions in Figure 2B. F-5 peptide alone promoted skin cell migration *in vitro* and wound closure *in vivo* as effectively as full-length Hsp90α-WT.[32] These findings demonstrated that the N-terminal ATPase domain and the C-terminal dimer-forming and cofactor-binding domain are dispensable with regard to secreted Hsp90α's ability to promote wound healing. Finally, Zou et al.[40] compared amino acid substitutions between Hsp90α and Hsp90β (which has no extracellular functionality). Sequential site-directed mutagenesis allowed them to identify two evolutionarily conserved lysine residues, lys-270 and lys-277, in the Hsp90α subfamily that determine extracellular Hsp90α function. From zebrafish to humans, all Hsp90β subfamily members lack the dual lysine motif. However, substituting Hsp90β's corresponding amino acid residues with lysines converted Hsp90β to behave like Hsp90α *in vitro*. These authors concluded that the N-terminal intrinsic ATPase and the C-terminal dimerisation region determine the intracellular chaperone function, whereas lys-270 and lys-277 determine the extracellular function of the Hsp90 protein family.

REGULATION OF EXOSOME-MEDIATED SECRETION OF HEAT SHOCK PROTEIN-90ALPHA

Hsp90 proteins lack the signal peptide (SP) required for the classical endoplasmic reticulum–golgi protein secretory pathway. An alternative secretory pathway for molecules without SP is secretion through extracellular vehicles, such as exosomes. By comparing 43 intracellular signalling pathways for their potential roles in regulation of exosome secretion in human keratinocytes, Guo et al.[45] identified the proline-rich Akt substrate of 40 kDa (PRAS40) as a pivotal signalling protein that connects environmental stress cues to exosome secretion machinery. This finding of PRAS40's new role in exosome secretion is a significant departure from the understanding of PRAS40 established in the last decade.

Most previous studies focussed on PRAS40's role in insulin signalling, as well that of other growth factors, in the mammalian target of rapamycin (mTOR) pathway (specifically mTORC1), which regulates cell metabolism, protein synthesis, and cell growth.[46-51] These studies showed that, in growth-arrested cells, PRAS40 binds to mTORC1 via the raptor subunit and inhibits the kinase activity of the complex. Insulin stimulation activates Akt kinase via threonine-308 (Thr-308) phosphorylation. The Thr-308-phosphorylated Akt kinase in turn phosphorylates PRAS40's C-terminus on Thr-246. Thr-246-phosphorylated PRAS40 dissociates from mTORC1, resulting in activation of mTORC1. The freed PRAS40 then (re-)associates with 14-3-3. However, PRAS40's mechanism of action here does not explain the results of many other studies, which demonstrate that PRAS40 plays a positive role in tissue repair and tumourigenesis.[52-56] The study by Guo et al.[45] demonstrated that activated PRAS40 has little to do with the mTOR pathway, because the authors failed to detect any significant activation of mTORC1 in PRAS40-depleted cells. Instead, PRAS40 knockdown (KD) or PRAS40 dominant negative (DN) mutant overexpression blocks tissue injury signaling, including TGFα, hypoxia, and H_2O_2, and oncogene-induced exosome secretion in a variety of normal and tumour cells. Site-directed mutagenesis and gene rescue studies showed that Akt-mediated activation of PRAS40 via Thr-246 phosphorylation is both necessary and sufficient to cause exosome secretion, without affecting the endoplasmic reticulum–golgi pathway. Identification of PRAS40 as a linker protein paves the way for understanding how stress regulates exosome secretion under pathophysiological conditions, such as wound healing.

THE TWO MAJOR BIOLOGICAL FUNCTIONS OF SECRETED HEAT SHOCK PROTEIN-90ALPHA

Using purified recombinant Hsp90α proteins/peptides, two important biological functions of extracellular Hsp90α have been uncovered in both *in vitro* cell culture and *in vivo* animal models: protection of cell survival under hypoxic conditions and promotion of cell motility during wound closure. First, using a hypoxic model as an example of microenvironmental stress, cells were observed to secrete Hsp90α to prevent hypoxia-induced apoptosis.[57,58] This finding is consistent with observed cellular behaviors in both physiological wound healing and cancer pathogenesis. During wound healing, cells in the hypoxic environment of the wound edge must remain viable prior to engaging in the repair process. Hypoxia and hypoxia-inducible factors (HIF)-1α are presently the best-characterised upstream stress cues that trigger Hsp90α secretion in both normal and tumour cells.[36,59,60] The second important biological function of extracellular Hsp90α protein is its novel ability to act as a motogen without promoting mitogenic activity. Recombinant Hsp90α promotes migration, but not proliferation, of a wide range of cell types in the total absence of serum factors or any other exogenously supplemented large molecules.[32,37]

Tsen et al.[43] demonstrated that the pro-survival and pro-motility functions of extracellular Hsp90α use a common signalling transduction pathway. According to their study, Hsp90α binds to subdomain II in the extracellular portion of the LRP-1. The NPVY, but not NPTY, motif in the cytoplasmic tail of LRP-1 connects Hsp90α signalling to Ser-473 (but not Thr-308) phosphorylation in Akt kinases, leading to enhanced cell migration. They further demonstrated that individual KD or knock-out of Akt1, Akt2, or Akt3 blocked extracellular Hsp90α signalling and wound healing *in vitro* and *in vivo*.[43] Secreted Hsp90α's promotion of cell survival and cell motility have been further verified in a distinct wound healing model. Bhatia et al.[41] assessed the pro-survival effect of extracellular Hsp90α using a unique pathological burn wound process called 'secondary burn wound progression', in which the wound expands horizontally and vertically from the initial site of trauma. If left untreated, cells in the expanded areas soon die of necrosis, apoptosis, or both, due to ischaemia, infection, and accumulation of toxic metabolites. These authors showed that topical application of recombinant Hsp90α dramatically reduces the degree of secondary burn wound progression by preventing heat-induced cell apoptosis. Additionally, topically applied recombinant Hsp90α protein strongly stimulates wound closure by promoting keratinocyte migration-driven re-epithelialisation in mouse and pig wounds.[32,34] Identification of these two functions of extracellular Hsp90α provides strong mechanistic support for currently ongoing therapeutic development of recombinant Hsp90α as a new treatment for various kinds of skin wounds.[35] These two findings are depicted in Figure 2C. Based on these findings, and those of studies previously discussed, Hsp90α is not an essential intracellular chaperone and is only used under stress, such as by an injured tissue.

WHAT MAKES SECRETED HEAT SHOCK PROTEIN-90ALPHA A BETTER WOUND HEALING AGENT THAN GROWTH FACTORS?

The authors have identified three unique properties of extracellular Hsp90α that are absent in conventional growth factors. First, extracellular Hsp90α is a common pro-motility factor for all three types of human skin cells involved in wound healing because all three cell types express a compatible level of LRP-1, the receptor required for extracellular Hsp90α signalling.[37] Following skin injury, keratinocytes migrate laterally to cover the open wound, and dermal fibroblasts and microvascular endothelial cells migrate inwardly to remodel the damaged tissue and re-establish a blood supply. Ideally, a single factor-based wound-healing agent should recruit all three types of skin cells into the wound bed. Secreted Hsp90α demonstrates this unique activity. Second, even in the presence of TGFβ3, extracellular Hsp90α remains equally effective in promoting migration of all three types of human skin cells. To the author's knowledge, secreted Hsp90α is the first molecule identified that can override the inhibitory effects of the TGFβ family of cytokines. Third, all forms of diabetes are characterised by chronic

hyperglycaemia, which is believed to be one of the major causes of delayed wound healing in diabetic patients. A reported mechanism of hyperglycaemia-induced wound healing impairment is destabilisation of HIF-1α protein, the key regulator of Hsp90α secretion.[61,62] While hyperglycaemia blocks hypoxia-induced and serum-stimulated human dermal fibroblast migration, extracellular Hsp90α not only enhances hypoxia-driven migration in normal glycemic conditions, but also 'rescues' migration of cells cultured in hyperglycaemic conditions.[32] In this case, extracellular Hsp90α may be promoting diabetic wound healing by bypassing HIF-1α down-regulation and jumpstarting migration of cells that could not otherwise respond to environmental hypoxia.

Keratinocyte migration occurs almost immediately following skin injury, whereas inward migration of dermal cells is not detected until 4 days afterward.[7] During wound healing, cell migration precedes cell proliferation; when a cell is actively migrating, it cannot proliferate simultaneously. As cells at the wound edge move toward the wound bed, they leave 'empty space' between themselves and the cells behind them, which subsequently begin to proliferate after losing contact inhibition with the front of travelling cells.

The stimuli responsible for this subsequent stage of cell proliferation are likely plasma growth factors that have diffused from surrounding unwounded blood vessels, where TGFβ levels are low or undetectable. The role of secreted Hsp90α leads and expedites the initial wound closure event, enhancing motility to prevent infection, water loss, and severe environmental stress. This understanding is schematically depicted in Figure 3.

This concept, together with Figure 1, frames the argument that the driving force during early wound closure comes from keratinocyte-secreted Hsp90α, and not growth factors, which fail to overcome the three biological hurdles, are short lived, and are unable to reach the wound bed due to haemostasis.

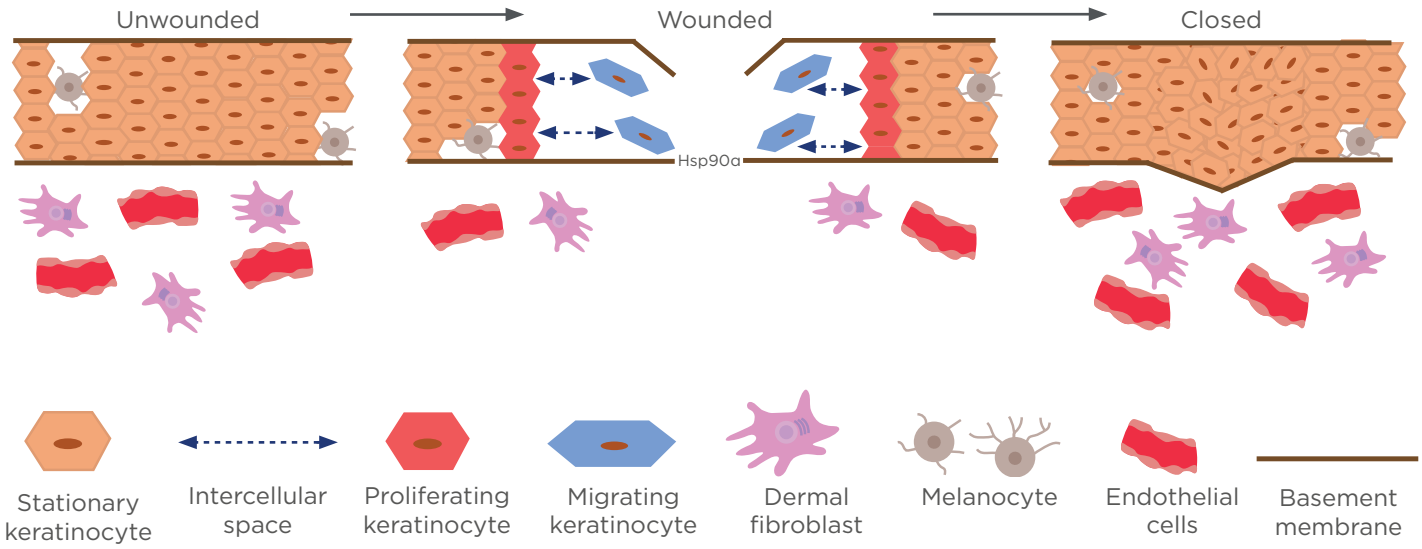

Figure 3: A model for migrating and proliferating keratinocytes around the wound edge.

In unwounded skin, cell–cell contact inhibition prevents both migration and proliferation. In wounded skin, keratinocytes (blue) at the wound edge secrete massive amounts of Hsp90α into the wound bed. The concentration of secreted Hsp90α reaches micromolar ranges within hours and serves as a chemoattractant for keratinocytes and dermal cells to migrate inward. The front of migrating keratinocytes creates space between themselves and the stationary keratinocytes behind them, resulting in the loss of contact inhibition that triggers the proliferation of the stationary keratinocytes to fill the gap. Together, these processes result in initial wound closure.

Hsp90: heat shock protein-90.

IS EXTRACELLULAR HEAT SHOCK PROTEIN-90ALPHA A 'GENERAL REPAIR MOLECULE'?

Recent studies have confirmed the previously contended notion that Hsp90α is not a critical intracellular chaperone like Hsp90β. Absence of Hsp90α or its intracellular chaperone form has a limited impact on mouse development,[39,62] whereas Hsp90β knockout is lethal to developing embryos.[63] At the cellular level, Zou et al.[40] have recently shown that CRISPR/Cas9 knockout of Hsp90β led to cell death, whereas knockout of Hsp90α had little effect on cell viability. At the signaling level, Jayaprakash et al.[57] reported that, under hypoxic conditions or conditions lacking serum factors, Hsp90β, but not Hsp90α, binds to the cytoplasmic tail of LRP-1 and stabilises the receptor at the cell surface. Hsp90α, but not Hsp90β, is then secreted by the same cells into the extracellular space, where it binds and signals through the LRP-1 receptor to promote cell motility, leading to wound closure. Regardless, the hypothesis that Hsp90α was designed by nature as a 'general repair' molecule is limited to indirect support and speculation, but the authors' research has led them to view the large stockpiles of Hsp90α distributed throughout our bodies as something like fire stations strategically dispersed around a city; for the same reason that we build fire stations before fires occur, Hsp90α, unlike conventional growth factors, is pre-stored and locally released when nearby tissue is injured. In doing so, Hsp90α responds quickly and arrives equipped to extinguish TGFβ and hypoxia-induced inhibition. More work will be required to prove or disprove this hypothesis, but the authors look forward to exploring these new questions together, alongside the scientific community, with optimism and excitement.

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Using the Novel Approach of an Artificial Pancreas to Manage Type 1 Diabetes Mellitus in Pregnancy

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Abstract

Recent National Institute for Health and Care Excellence (NICE) guidelines suggest that insulin pump therapy should be used in pregnant women with Type 1 diabetes mellitus (T1DM) who do not achieve optimal glycaemic control with multiple daily injection (MDI) therapy. Furthermore, a landmark trial has confirmed that prospective continuous glucose monitoring (CGM) may be beneficial for women using both MDI and insulin pumps during pregnancy, with positive effects on neonatal outcomes. More recently, overnight use of an artificial pancreas (AP) with a model-predictive control algorithm has been shown to improve the amount of time women spend within the overnight glucose target range (3.5–7.8 mmol/L) during pregnancy. However, preliminary studies where the AP is used day and night have shown a high degree of interindividual variability in response to the intervention, and further randomised trials are needed to understand which women are suitable candidates for CGM, insulin pump, and AP technology. It is understood that improvements in maternal glycaemic control can minimise the risk of adverse neonatal outcomes. Given the substantial improvements in glycaemic control with AP use outside of pregnancy, the recent advances in AP technology provide hope that AP systems will improve the effectiveness of continuous subcutaneous insulin infusion and CGM during pregnancy. Further research is needed to evaluate whether AP can optimise glucose control and neonatal outcomes in T1DM pregnancy. This paper will discuss emerging technologies available for the management of T1DM in pregnancy.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) in pregnancy increases the risk of obstetric and neonatal complications associated with maternal hyperglycaemia.[1] These widely recognised complications include higher rates of congenital anomaly, macrosomia, shoulder dystocia, stillbirth, and neonatal death.[2] The primary goal for these women is to improve their glycaemic control, thereby improving maternal fetal outcomes.[3]

The physiological state of pregnancy is diabetogenic and therefore the additional hyperglycaemia of T1DM can have significant adverse effects.[4] The decreased response to insulin observed during pregnancy has often been attributed to an increased level of progesterone,

oestrogen, prolactin, human placental-lactogen, and resultant 'increased placental-fetal glucose demands'.[4,5] As gestation progresses in T1DM, hormonal and other factors cause fluctuations in insulin requirements, making insulin dosing difficult to predict.[6] Further, women with T1DM are 3–5-times more likely to experience severe hypoglycaemia during early pregnancy compared with the period before conception, and some women, especially those with impaired awareness of hypoglycaemia, experience recurrent hypoglycaemia episodes throughout pregnancy.[7] It is hoped that diabetes technology will miminise the burden of hypoglycaemia.

PREVALENCE AND EPIDEMIOLOGY

In the UK, approximately 700,000 women give birth annually and 2–5% of these pregnancies are complicated by diabetes.[8] Of these, around 88% involve gestational diabetes, 5% involve Type 2 diabetes mellitus, and 7% involve T1DM.[8] The rate of neonatal death (death of a live-born infant up to 28 days after delivery) in T1DM pregnancies across the UK is currently 8.1 in every 1,000 births, 2–5-times higher than the non-diabetic population. This remains unchanged from 2002.[1] Across the UK, the only pregnancy outcome to significantly improve since 2002 is the rate of stillbirth (fetal loss after 24 weeks' gestation), which is 2.5-times lower than the figure obtained in the 2002–2003 Confidential Enquiry into Maternal and Child Health (CEMACH) (10.7 versus 25.8 in every 1,000 births, p=0.0012).[1] This decrease is believed to be largely due to changes in obstetric management, which now recommends more timely induction of labour for women with diabetes, since there have been no improvements in glycaemic control during T1DM pregnancy.[9]

Despite the advances in diabetes management and widespread use of insulin analogues, in the UK, only around 14.3% of women with T1DM manage to achieve adequate glycaemic control during the first trimester.[1] Moreover, continuous glucose monitoring (CGM) data demonstrate that women with T1DM are spending an average of 12 hours per day in the target glucose range during early pregnancy.[11]

MEASUREMENT OF GLYCAEMIC CONTROL DURING PREGNANCY

Continuous Glucose Monitoring

CGM measures the level of glucose in the interstitial fluid through a subcutaneous sensor and transmits the information via radio frequency to a data display receiver or smartphone, providing real-time glucose monitoring. For masked or professional GCM, the glucose data is uploaded from a docking station and only available to patients, clinicians, and/or researchers retrospectively. CGM provides between 96 and 288 glucose measurements per day, with in-depth information on glucose fluctuations and trends over time.[12] This information enables users to visually appreciate the feedback that their diet and physical activity has on their glycaemic control and can serve as an important educational resource.[12]

At present, there are four main types of CGM systems available: retrospective, real-time, on-demand, and implantable. Retrospective CGM continuously records information regarding glycaemic control; however, this information is not visible to the user in real-time and is instead downloaded for retrospective evaluation of glucose trends to facilitate lifestyle and pharmacological modifications.[13] Given the frequency of fluctuations in glucose levels during pregnancy, prospective adjustments to insulin delivery based on retrospective glucose information will not adequately address all women's needs. As such, the applicability and usefulness of retrospective GCM during pregnancy is limited.

Real-time CGM provides users with immediate feedback on their blood sugar levels, allowing users to see the impact of exercise, sickness, and carbohydrate intake on their glycaemic control.[14] Flash glucose monitoring does not require calibration with capillary glucose testing, and uses a 14-day sensor that, when scanned, provides users with their current glucose level (thus referred to as on-demand monitoring) and a graph of glycaemia since their previous scan.[15] Furthermore, the U.S. Food and Drug Administration (FDA) has recently approved the use of the implantable Eversense® CGM device (Senseonics, Germantown, Maryland, USA).[16]

Available in Europe and the USA, this device has a fluorescence-based sensor that is implanted subcutaneously. A transmitter worn above the sensor sends information to a mobile phone app that displays the glucose values and their trends. Compared with current CGM devices, which need to be replaced up to every 14 days, the Eversense lasts up to 3 months.[16] Eversense has not yet been evaluated for use in pregnancy, but it is assumed that its accuracy during pregnancy would be comparable to outside pregnancy, as per other sensors.[17]

Two early trials of CGM during pregnancies complicated by T1DM suggested no benefits of CGM over traditional monitoring techniques. A 2013 study by Secher et al.[18] showed no improvement in glycaemic control or neonatal outcomes when real-time CGM was used intermittently (on average five times) during pregnancy. The subsequent 'Glucomums' randomised controlled trial,[19] comparing retrospective CGM with conventional treatment, also failed to show any improvement in glycaemic control or neonatal outcomes with CGM use. In both studies, both real-time and reterospective CGM were used intermittently. Outside pregnancy, it has been established that real-time CGM needs to be worn >70% of the time for a significant improvement to be seen.[20]

More recently, the results from the large, robust, CONCEPTT study[21] were published. This multicentre, open-label, randomised controlled trial allocated 215 pregnant women and a further 110 who were planning pregnancy to CGM or capillary glucose monitoring alone. The results demonstrated a modest improvement in HbA1c with CGM use (-0.19%, 95% confidence interval [CI]: -0.34–-0.03; p=0.0207) and more time-in-target range (68% versus 61%; p=0.0034). This translated into significant clinical improvements in the CGM group, with a lower incidence of large for gestational age (LGA) infants (odds ratio [OR]: 0.51, 95% CI: 0.28–0.90; p=0.0210) and fewer neonatal intensive care unit (NICU) admissions over 24 hours (0.48; 0.26–0.96; p=0.0157).[21]

The NICE guidelines (Section 1.3.18, revised in 2015)[3] recommend real-time CGM use for women with problematic severe hypoglycaemia, unstable blood glucose levels (to minimise variability), and to gain information about variability in blood glucose levels. However, only 16% of T1DM women achieve the NICE glycaemic control target of HbA1c <48 mMol/Mol (6.5%) in early pregnancy and almost all have unstable glucose levels.[1] As of July 2018, NICE announced that, based on findings from the CONCEPTT trial, it is planning a partial update of its guidelines for CGM use in T1DM pregnancy.[22]

Currently, CGM is not subsidised for pregnant women on the NHS. The costs of real-time monitoring over 28 weeks (from 10–38 weeks gestation) can be up to £2,500.[23] However, this needs to be offset against the cost of not subsidising the technology. In the CONCEPTT study, the rate of NICU admissions >24 hours was 27% in CGM and 43% in the control group (OR: 0.48, CI: 0.26–0.86; p=0.0157).[21] The mean cost of hospitalising a neonate in a level 3 NICU is >£17,861.[24] This benefit alone, notwithstanding the other clinical benefits, provides a compelling basis for performing cost-benefit analyses for GCM during T1DM pregnancies.

In addition to using CGM as a tool for guiding insulin dosage, there is a move to use GCM data as a measure of the adequacy of glycaemic control. During pregnancy, there are physiological changes that render HbA1c a less effective measure of glycaemic control. For example, red blood cells during pregnancy have an increased affinity for glucose.[25,26] The CONCEPTT trial confirmed the clinical relevance of time-in-target as an appropriate outcome measure during pregnancy. A 7% increase in time-in-target range during pregnancy was associated with a significant (approximately 50%) reduction in neonatal complications.[21]

Continuous Subcutaneous Insulin Infusion (Insulin Pumps)

Outside of pregnancy, it has been widely established that continuous subcutaneous insulin infusion (CSII) is safe and is associated with improved glycaemic control.[27,28] However, the benefits of the technology during pregnancy are less well established.

CSII is thought to be a more 'physiologic' method of insulin delivery because it is closer than other forms of insulin delivery to the pattern of insulin secretion from the pancreas.[29] When purchased privately, CSII can cost between £2,000 and £3,000 in the UK.[30] These pumps can be programmed to have different basal rates

at different times of the day according to the need of the individual user, which is a key advantage over multiple daily injection (MDI) therapy.[31] Notably, in a pre-specified secondary analysis of CONCEPTT data, it was found that MDI users had a larger decrease in HbA1c and were more likely than CSII users to achieve target HbA1c.[32] The offspring of MDI users had fewer NICU admissions and reduced levels of gestational hypertension.[32] These findings should be interpreted with caution due to baseline differences in maternal smoking and hypertension, but they suggest that CSII implementation during pregnancy may be suboptimal.

A Cochrane Review[33] of four studies performed in 2007 that compared CSII with MDI during pregnancy concluded that there were no differences in neonatal outcomes or glycaemic control with use of the technology. However, the first study did not include any information about the randomisation process and the second study recruited a skewed sample population of highly educated, highly motivated participants.[33] The possibility of bias in these studies can therefore not be excluded. Furthermore, the sample sizes were small and lacked statistical power to detect between group differences in obstetric and neonatal outcomes.

It has been postulated that the improvement in glycaemic control when using CSII is due to the ability of the technology to facilitate more frequent fine-tuning of the dosages of insulin.[27] Yet, due to conflicting evidence, CSII is not routinely recommended during pregnancy. The NICE guidelines now reflect this uncertainty by recommending its use in pregnancy when adequate glycaemic control is not obtained with MDI use.[3]

Sensor Augmented Pump Therapy

Sensor augmented pump therapy (SAP) is a combination of CGM and CSII therapy that allows for close monitoring of glucose levels and tighter adjustments of insulin dosing.[34] The CGM and insulin pump communicate in real-time to store and transmit information regarding glycaemic control. This information can be uploaded to the internet for use by people with diabetes and their clinicians.[34]

The potential benefits of SAP were demonstrated in the STAR 3[35] trial that showed that the addition of CGM to CSII in non-pregnant adults resulted in decreased HbA1c levels and less time spent in a hypoglycaemic state. In CONCEPTT, participants using CSII had a significant improvement in the percentage of time-in-target with the addition of CGM, suggesting that CGM improves the effectiveness both of MDI and of CSII.[21] A pilot study in pregnant women with T1DM compared SAP with CGM and demonstrated a lower HbA1c level in the SAP group (6.52% versus 6.82%; $p<0.05$).[36]

Closed-Loop Insulin Delivery Device (Artificial Pancreas)

The closed-loop insulin delivery device, or artificial pancreas (AP), is an automated insulin delivery system comprised of a CGM, a CSII, and an algorithm that amalgamates real-time information from the CGM to adjust insulin delivery (Figure 1).[37,38]

Outside of pregnancy, the AP has been shown to be effective at improving glycaemic control and quality of life in children and adults without increasing rates of hypoglycaemia, when compared to conventional treatment or SAP.[37,39,40] A recent meta-analysis of 24 randomised control trials (N=585) demonstrated that, outside of pregnancy, the AP improved time-in-target by 3 hours compared with stand-alone pump therapy (target range: 3.9–10.0 mmol/L).[41] AP also reduced the amount of time spent in a hypoglycaemic state by almost 50% (from approximately 5.0% to 2.5%).[41] In 2016, the FDA approved the use of Minimed™ 670G (Medtronic, Dublin, Ireland), which uses a proportional-integrative-derivative (PID) control algorithm, as the first commercially available AP.[42]

Minimed 670G is a hybrid automated system that replaces basal insulin doses, but the device still requires the user to self-administer prandial boluses via the insulin pump. The control algorithm that has been assessed during pregnancy is the model-predictive control (MPC) algorithm.[17,43] This algorithm "predicts future glucose excursions based on patients' glycaemic responses to insulin and meals".[44] There are a number of different algorithms that have been trialed in the AP. In their 2017 meta-analysis of 24 studies, Weisman et al.[41] demonstrated

that the improvement in time-in-target with the AP compared with CSII is approximately 12.6%, regardless of the algorithm used. PID was associated with a slightly lower time-in-target compared to fuzzy logic and MPC algorithms, although this was not statistically significant.[41]

The first two Closed-Loop In Pregnancy (CLIP) studies, CLIP 01 and 02, were randomised, crossover pilot studies that demonstrated the safety and efficacy of implementing a MPC algorithm in pregnancy.[17,43] The subsequent CLIP 03 study was an open-label, randomised crossover study comparing overnight closed-loop therapy with SAP in 16 pregnant women with T1DM.[45] The women completed 4 weeks of closed-loop therapy (intervention) and 4 weeks SAP (control) in random orders, followed by a continuation phase, in which the closed-loop system was used day and night until discharge from hospital after delivery.[45]

The study demonstrated a significant improvement in the percentage of time overnight spent in the target blood glucose range of 3.5–7.8 mmol/L with use of the AP (74.7% versus 59.5%; absolute difference: 15.2% points; 95% CI: 6.1–24.2; p=0.002). The mean overnight glucose level was also lower with AP use compared with the control (6.6 versus 7.4 mmol/L; p=0.009), and AP use was not associated with an increase in hypoglycaemia frequency.[45] There was no statistically significant improvement in HbA1c,[45] although this is unsurprising given the relatively short study period and the limitations of using HbA1c measurements in pregnancy.[46] The system was able to cope well with the physiologic challenges of pregnancy, including antenatal steroid administration, labour, and anaesthetics.[45]

Although the observed improvements in glycaemic control were promising, there was a higher than expected number of adverse obstetric and neonatal outcomes in this study. Thirteen of the fetuses were LGA, 12 required a NICU admission, 5 women had pre-eclampsia, and 15 women delivered by caesarean section.[45] However, the study population were high-risk and included a number of women with a poor obstetric history. Further analyses of neonatal outcomes are not possible because the randomised intervention lasted only 4 weeks and the crossover trial was not designed to assess neonatal outcomes.

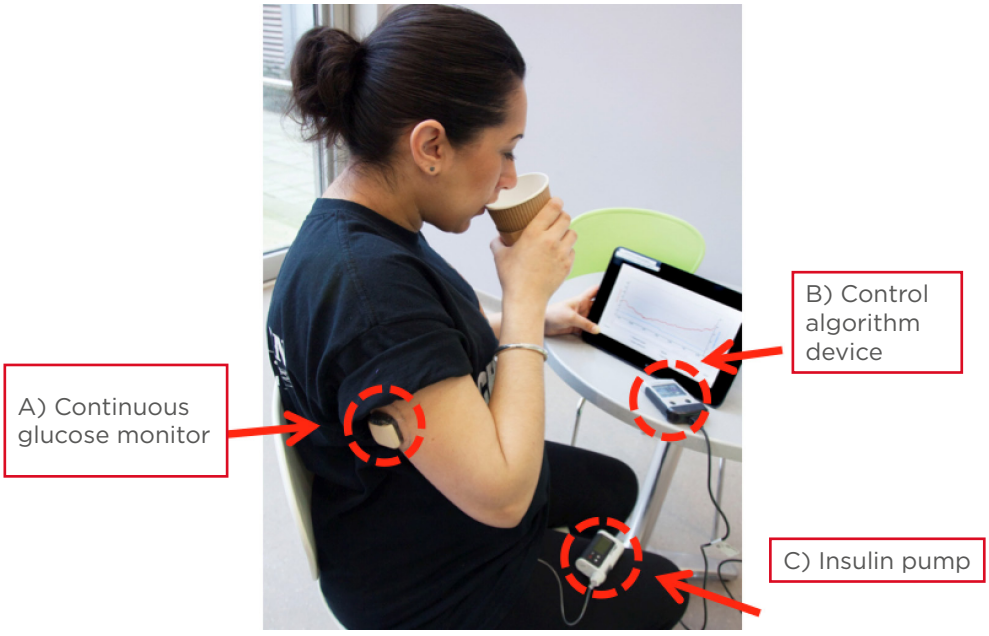

Figure 1: Study participant using the closed-loop insulin device

Components of a closed-loop system: A) continuous glucose monitor (sensor, transmitter, and receiver); B) a control algorithm device; and C) an insulin pump.

Adapted from Stewart.[38]

The most recent study, CLIP 04,[47] had an almost identical study design to CLIP 03; however, the participants used closed-loop day and night during the intervention phase, rather than only overnight as in CLIP 03. Participants in CLIP 04 showed no significant difference in the proportion of time spent in the target day-and-night regions with AP use (62.3% versus 60.1%, p=0.47), although fewer hypoglycaemic episodes occurred with AP use (time spent <3.5 mmol/L, 1.6% versus 2.7%, p=0.02).[47]

The rates of adverse neonatal outcomes in CLIP 04 were more in line with clinical expectations than in CLIP 03 (2/16 women had pre-eclampsia, 7/16 infants were LGA, and 11/16 were admitted to the NICU).[47] However, it is noteworthy that a number of factors may have resulted in the varied outcomes of CLIP 03 and 04. Firstly, CLIP 04 examined AP use during the day as well as overnight. It is far more challenging to achieve ideal glycaemic control during the daytime, when meals, snacks, exercise, and premeal bolusing are factors.[47] Moreover, in CLIP 04, during the SAP control phase, participants spent 60% of the time within the pregnancy target range of 3.9–7.8 mmol/L (during day-and-night). This 'control' rate is substantially higher than in normal control groups outside of pregnancy, and was comparable to or higher than the degree of control achieved previously in other studies of AP.[37,39] This minimises the potential for further improvement with the AP.

In CLIP 04, there was a high degree of inter-individual variability with some women demonstrating improvements in glycaemic control with the intervention and others having no improvement with the intervention.[47] Notably, a broad population range were recruited in CLIP 04 with the women coming from a range of educational backgrounds and socio-economic statuses and having varying levels of baseline glycaemic control. Furthermore, >80% of participants were sensor naïve.[47] In a sample size of 16 patients, these factors can significantly influence the results of the studies.

There were a number of limitations to CLIP 03 and 04. The relatively short 4-week study duration may not be sufficient to train device-naïve participants. Moreover, the cross-over study design may not have been ideal for participants with variability in their lifestyle. However, overall, these four proof-of-concept studies investigating day-and-night closed-loop insulin delivery demonstrated that the technology may be able to safely achieve acceptable glycaemic control during pregnancy.

Recently, a prespecified secondary analysis of the data from CLIP 03 and 04 was performed.[48] It aimed to identify the baseline characteristics of the study participants that could predict a positive response to the AP. Women with lower baseline HbA1c had a more significant biomedical improvement (p=0.014) than women with a higher baseline HbA1c.[48] Although the exact reason for this is unclear, researchers postulated that the same factors that precipitated optimal preconception glycaemic control, such as high levels of health motivation and health literacy, facilitated the optimal implementation of closed-loop. Age, BMI, duration of diabetes, location of diabetes treatment, and previous experience with CSII had no effect on the treatment effect.[48]

Women were willing to bear the burdens and limitations of the device, including frequent malfunctions and physical bulkiness, if they perceived that it improved their biomedical control and quality of life.[48] Reported short comings of the technology included frequent alarm malfunctions, slow connections between the CGM and the CSII, and the cumbersome nature of carrying around a device. In contrast, the same women noted improved control, a strong sense of mental freedom resulting from the lesser requirement to calculate insulin dosages, improved sleep, and a reduced sense of hypo and hyperglycaemia-related anxiety with the AP.[48]

In 2019, a parallel-arm, randomised controlled trial of 124 women across 10–12 NHS antenatal diabetes centres in England, Scotland, and Northern Ireland will be conducted. The open-label AiDAPT trial will assess the clinical efficacy of the AP on glycaemic control in pregnant women with T1DM. This trial will use the Dexcom® G6 CGM (Dexcom, San Diego, California, USA), which does not require users to perform fingerprick calibrations.[49]

CONCLUSION

Despite new technologies and recommendations about how best to manage T1DM in pregnancy, obstetric and neonatal outcomes remain

suboptimal. CGM, insulin pumps, and the AP have shown promise in the management of the disease; however, the evidence regarding the use of these technologies is rapidly evolving as the devices develop and it is challenging for healthcare professionals to remain up-to-date with this ever-changing landscape.

With the advent of each new promising diabetes technology, there has been hope for a product that will achieve optimal glycaemic targets with minimal effort and disruption to daily life. However, the benefit of CSII has been modest at a population level and further research is required before the efficacy and cost-effectiveness of SAP can be robustly concluded.[38] Given the complexity of diabetes management, it is likely that no single technology will be able to cure the condition. Therefore, any technology that manages to safely and efficaciously improve glycaemic control and quality of life of pregnant women with T1DM is worth considering. At present, considering the data from CONCEPTT, the authors suggest implementing GCM into routine care of pregnant women with T1DM. The addition of CSII should be considered on a case-by-case basis, with clinicians considering baseline glycaemic control, patient preference, and affordability.

The AP represents an exciting frontier in the management of T1DM in pregnancy. In studies to date, AP has shown an ability to cope with the physiologic challenges of pregnancy and may lead to improvements in diabetic outcomes such as an increased time spent in target. The technology was well received by participants and almost all of the women in the AP trials elected to continue using it after the crossover phase of the study, rather than returning to their previous therapy.[45,47] However, further evidence from randomised controlled trials is needed to understand whether AP can help to bridge the gap between clinicians expectations of tight glucose control during pregnancy and the reality of day-to-day glucose excursions experienced by patients.

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