An international perspective on the future of systemic sclerosis research.

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Abstract

Systemic sclerosis (SSc) remains a challenging and enigmatic systemic autoimmune disease owing to its complex pathogenesis, clinical and molecular heterogeneity and the lack of effective disease-modifying treatments. Despite a century of research in SSc, the interconnections between microvascular dysfunction, autoimmune phenomena, and tissue fibrosis in SSc remain unclear. The absence of validated biomarkers and reliable animal models complicates diagnosis and treatment, contributing to high morbidity and mortality. advances, such as single-cell RNA sequencing, next-generation sequencing, spatial biology, transcriptomics, genomics, proteomics, metabolomics, microbiome profiling and artificial intelligence, offer new avenues for identifying the early pathogenetic events which, once treated, could change the clinical history of SSc. Collaborative global efforts to integrate these approaches are crucial to develop a comprehensive, mechanistic understanding and enable personalized therapies. Challenges include disease classification, clinical heterogeneity and the establishment of robust biomarkers for disease activity and progression. Innovative clinical trial designs and patient-centred approaches are essential for developing effective treatments. Emerging therapies, including cell-based and fibroblast-targeting treatments, show promise. Global cooperation, standardized protocols and interdisciplinary research are vital for advancing SSc research and improving patient outcomes. The integration of advanced research techniques holds the potential for significant breakthroughs in the diagnosis, treatment and care of individuals with SSc.

[H1] Introduction.

Sir Winston Churchill's definition of the Soviet Union as "*a riddle, wrapped in a mystery, inside an enigma*" could also apply to systemic sclerosis (SSc, also known as scleroderma. The pathogenesis of SSc, lack of animal models, clinical heterogeneity, absence of validated biomarkers, difficult classification, problematic monitoring of treatment response and lack of well-designed randomized control trials contribute towards the complexity of SSc [1,2]. Consequently, diagnosis is often delayed, the assessment of disease activity is challenging, there is a lack of reliable disease-modifying drugs, and morbidity and mortality are high, resulting in a substantial [burden on patients and the healthcare system [3,4]. Despite more than a century of clinical and experimental investigations into SSc since the first descriptions of the disease, [5,6] the precise relationship among the hallmark features of SSc — microvascular dysfunction, autoimmune phenomena and pathological tissue fibrosis — remains elusive. It is unclear whether these events are interlinked, triggered by the same or different agents, or occur as a cascade. Additionally, the heterogeneous nature of SSc raises questions about whether it is one disease with distinct subsets and stages or a collection of closely related diseases with similar symptoms (Figure 1).

As no animal model encompasses all features of SSc, insights gained from animal studies remain limited [7] Most evidence suggests that vascular abnormalities or immunological dysregulation occur at early stages of the disease and precede fibrosis. This model of pathogenesis, however, has not translated into therapeutic

advances. Most treatment strategies target single-organ manifestations, often improving quality of life and survival but falling short of being disease-modifying therapy. Unclear definitions, variability in patient subsets, small and underpowered studies and difficulties in obtaining patient samples contribute to these challenges. Examples include studies with tocilizumab (anti-IL6 receptor therapy), abatacept (CTLA-4 co-stimulation blocker), and pan-PPARγ agonists which failed to meet the primary endpoints [Although the anti-fibrotic agent nintedanib was shown to have beneficial effects for patients with interstitial lung disease (ILD) [8], and some studies with TGFβ inhibitors showed promising effects [9,10], much remains to be done to effectively manage all the manifestations of SSc.

There has been substantial progress in molecular and cell biology and cellular immunology, coupled with technological breakthroughs in next-generation sequencing, single-cell RNA sequencing, spatial transcriptomics, proteomics and metabolomics. Integrating these research technologies into SSc research requires computational biology, statistical and artificial intelligence (AI) to properly analyse the data generated in studies using them and to link them to clinical information. These unprecedented recent developments now provide the field with a unique opportunity to integrate the new approaches into SSc research in a systematic fashion to generate a more detailed mechanistic understanding of disease pathogenesis and to make personalised medicine in SSc a reality by identifying specific and effective treatments tailored for individual patients. Organized global cooperation is a prerequisite for the success of such an endeavour. With this aim, an international consensus workshop was held in Portonovo (Ancona) Italy in October 2023 . The goals of the workshop were to provide a foundation for bringing the international SSc research community together, to develop a common understanding of the disease pathobiology, to explore innovation in clinical trial designs and outcome measures, as well as to educate healthcare professionals, patients, the general public, regulators, industry leaders and policymakers (Figure 2).

[H1] The complexity of SSc and organ-based complications.

SSc is an acquired autoimmune disease with a strong female sex bias and is characterized by vascular damage and immunological abnormalities that lead to immune dysfunction, autoantibody production and the development of skin and internal organ fibrosis [1-3]. Its complexity arises from disease heterogeneity, the involvement of multiple organ systems and a blend of inherited, environmental and lifestyle factors. Recent research deploying advanced omics and analytic approaches has unveiled novel pathways, cell types, circuits and mechanisms involved in SSc, offering new therapeutic targets and insights into disease pathogenesis SSc is highly heterogeneous and can be readily separated into two major disease subsets, limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc), according to the extent of skin involvement (Figure 1) [1-3]. Other aspects of disease diversity include variations in clinical manifestations, disease progression, organ involvement, treatment response and molecular heterogeneity [11], thus emphasising the need for personalized treatment approaches.

Advances in genomic and proteomic platform technologies have accelerated the identification of molecular pathways underlying SSc pathogenesis. In addition to well-known signalling pathways (such as the TGFβ, CCN, platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor binding protein, IL-6 and IL-31 signalling pathways) novel mechanisms have been implicated, such as Notch, Hedgehog, Wnt–β–Catenin, CXCL4, and various extracellular matrix (ECM) remodelling pathways [12-16]. Understanding the interconnections between these pathways is critical for identifying new therapies. The pathology of SSc is traditionally defined by vascular dysfunction, inflammation, autoimmunity and hyperactivation of myofibroblasts. Recent approaches including single-cell and spatial analysis, have extended the knowledge of the cell types that are involved in SSc and the communication of these cells with each other and the ECM, known to be a major contributor in disease pathogenesis [17-19]. Researchers have uncovered novel aspects of fibroblasts and myofibroblasts that highlights the plasticity of these cells. The fibrotic tissue in patients with SSc contains multiple transcriptionally distinct fibroblast subsets [17], some of which have critical] roles

in the initiation, progression and persistence of pathology (Figure 3). Recent studies have also focused on the pathogenetic role of the microbiota of the skin, lungs and gut; however, establishing the effects of microbial dysbiosis in different organs on the initiation and progression of SSc features remains challenging [20,21].

Genomic studies have identified nearly 30 loci associated with SSc, highlighting the contribution of immune cell activation, type I interferon signatures, cytokine signalling, inflammation, apoptosis and autophagy to the disease process [22]; these studies also provide insight into vascular and ECM fibrotic pathology. The assessment of shared genetic factors and cross-phenotype genome-wide association studies have uncovered new pathways by identifying shared genetic factors between SSc and other autoimmune diseases [23]. This overlap provides opportunities to extend applications for specific drugs, potentially accelerating the development of effective therapies for SSc. Future directions in SSc genomics include trans-ethnic genome-wide association studies, whole-genome sequencing, studying structural and non-coding RNA variants and epigenetic studies. The integration of genomic data with epigenomic, transcriptomic and proteomic data can help elucidate the functional effect of genetic variants [24-25]. Deciphering the interplay between genetic predisposition, environmental exposures and social factors will increase the understanding of SSc pathogenesis. Environmental factors, such as occupational exposures, infections and lifestyle factors can increase disease risk and could trigger onset and influence progression of SSc.

Besides skin involvement and Raynaud phenomenon, organ-based complications are a key feature in SSc and can involve several internal organs, including the kidney, gastrointestinal tract and cardiopulmonary system [1]. The underlying pathophysiology seems similar, with some organ-specific pathogenic mechanism(s) Skin involvement in SSc can cause substantial morbidity; management of skin manifestations consists of vasodilators and immune-based therapies [1]. Gastrointestinal tract involvement is common and represents an important unmet clinical need, with underlying molecular and cellular alterations still being investigated. SSc-associated pulmonary complications (pulmonary arterial hypertension (PAH) and ILD) are complex, life-threatening and require early recognition, accurate diagnosis and comprehensive management [26]. Recent research into pulmonary vascular disease has provided genetic insights and new concepts regarding the pathophysiology of SSc-associated PAH [27-28]. Around 5% of patients with SSc have pathological coding variants in PAH-related genes. Advances in the treatment of PAH focus on vasodilation and targeting the underlying pathobiology of the disease. New therapeutic agents, such as sotatercept, an activin signalling inhibitor, show promise but raise concerns about adverse effects such as vascular malformations. Other potential treatments include small molecule tyrosine kinase inhibitors, poly (ADP-ribose) polymerase inhibitors, bromodomain-containing protein 4 inhibitors senolytics, elastase inhibition, stem cell therapy, autologous hematopoietic stem cell transplantation (HSCT) and chimeric antigen receptor (CAR) T cell therapy [29-32].

In SSc-associated ILD (SSc-ILD), inflammation and fibrosis of the lungs can substantially impair lung function and, hence, quality of life, and is associated with high mortality. The course of SSc-ILD is highly variable and predicting individual disease progression is problematic owing to the lack of reliable biomarkers, which hinders the implementation of personalised therapy [1-3]. Current treatments for SSc-ILD focus on slowing disease progression, managing symptoms and improving patient outcomes. Typical treatments include immunosuppressants (mycophenolate mofetil, cyclophosphamide and rituximab) and new antifibrotic agents (nintedanib) [8, 29-33]. Additionally, in a subset of patients with SSc, treatment with tocilizumab can help manage symptoms and improve functional capacity [34-35]. Ongoing research continues to explore new therapeutic options and strategies to identify early events in the pathogenesis of SSc-ILD and better address SSc-ILD and its effect on patients. The use of the 2019 classification criteria [36] for the early identification of scleroderma renal crisis should facilitate early treatment with angiotensin-converting enzyme inhibitors and improve the prognosis of this rare but severe complication. Vasodilators used in PAH (endothelin receptor antagonists and prostacyclin analogues) have not demonstrated efficacy in this setting but could, in the future, along with complement inhibitors (eculizumab) help improve the prognosis of renal crisis in SSc. Heart

involvement is the third leading cause of death related to organ involvement in SSc; heart involvement in SSc represents an unmet clinical need as only symptomatic non-specific treatments are proposed for these patients. This cardiac involvement remains an understudied area of research in SSc [37]. Patients with **SSc** can undergo organ transplantation, particularly for the lungs, heart and, in some cases, the kidneys. Organ transplantation is considered for patients with severe, end-stage organ involvement that is refractory to other treatments [1-3].

The association of SSc with aging markers provides fresh insights into its pathogenesis [38-41]. Biological clocks indicate an apparent acceleration of aging in individuals with SSc, and cellular senescence is greatly augmented in affected organs [42]. Senescent cells, which are characterized by irreversible cell cycle arrest and the senescence-associated secretory phenotype, increase in abundance with age and correlate with chronic inflammation [43]. Senolytics and senomorphic therapies aim to eliminate or reduce the effect of senescent cells, but the reparative role of senescent fibroblasts requires careful investigation [44]. Accurately measuring biological aging and cellular senescence in SSc and understanding their contributions to pathogenesis can provide new therapeutic targets.

A compelling need exists to better understand the natural history of SSc and the various subtypes of this disease. Long-term, multicentre, and multinational longitudinal studies are crucial for capturing disease progression, identifying biomarkers and evaluating therapies. Although these studies present challenges, such as patient recruitment and standardizing data collection, the potential benefits outweigh these obstacles, offering improved patient outcomes and a deeper understanding of SSc.

[H1] Overcoming major barriers for research in SSc

Research in SSc faces several hurdles that hinder progress toward effective therapies; for example, the difficulties in defining and classifying SSc, the relative rarity and clinical heterogeneity of the disease, the complex heritability and mysterious aetiology and perhaps, most notably, the incomplete understanding of underlying molecular mechanisms. These factors and operational changes in outcome assessment , impede efforts to generate definitive evidence for altering clinical practice. Many of these issues are interconnected. Untangling the complex and dynamic temporal and pathogenic relationships linking fibrosis, vascular injury and immune activation remains elusive. A more complete understanding of the pathogenesis of SSc will probably emerge from a combination of both unbiased and hypothesis-driven approaches that address all three hallmark features: vascular, immunological and fibrotic changes.

The striking female bias in SSc, similar to many other autoimmune diseases, remains unexplained, with multiple competing, although not mutually exclusive, proposed underlying mechanisms. Exciting recent findings suggest a potential role for X-chromosome inactivation escape and Xist ribonucleoproteins [47]. Multiple studies have reported that the intestinal microbiome of patients with SSc is different from that of healthy individuals [48, 49]; however, the pathogenic role of gut dysbiosis, and the potential mechanisms involved, remain completely unknown and merit further study [50, 51].

As already noted, current animal models of SSc fail to capture all aspects of human disease. Alternate preclinical model systems, such as precision-cut skin slices and 3D organ cultures that are populated with multiple cell types (such as monocytes, fibroblasts or endothelial cells), might aid the efforts to better understand pathogenesis and for preclinical drug testing [52-57]. It is widely accepted now that improved disease models are essential for assessing the efficacy of new therapies and understanding drug interactions in clinical trials. These models are promising, especially when leveraging emerging technologies with single-cell level resolution, such as single-cell RNA sequencing, single-cell ATAC-sequencing and spatial transcriptomics [17,19,58-60]. Incorporating the potential environmental exposures and acquired genetic and/or epigenetic

changes [61-63] are expected to yield novel insights into pathogenesis and enable more predictive determination of efficacy of therapeutics. In addition, emerging interest in leveraging endogenous anti-fibrotic pathways offers new opportunities for boosting blunted or suppressed anti-fibrotic responses in SSc and other fibrosing diseases [64,65].

Detecting early-stage SSc remains an important challenge as this stage of disease could be the most responsive to disease-modifying therapies (Figure 1.). The VEDOSS (Very Early Diagnosis of Systemic Sclerosis) classification criteria help identify disease risk, but studies [66] show that autoantibodies often appear years before the onset of overt disease. Antibodies to Ro52, Ro60 and CENP-A are elevated decades before disease manifests and remain high, whereas anti-RNA polymerase III and anti-topoisomerase I antibodies increase progressively and more rapidly as clinical diagnosis approaches. Interestingly both Ro52 and Ro60 proteins are linked to interferon, and a type I interferon signature in monocytes has been found at the earliest phases of SSc before overt fibrosis, suggesting that it is also an early event in the pathogenesis of the disease [67-69]. This relationship should be explored in more depth since it can provide hints as to what triggers the disease. These findings suggest that early biomarkers could be crucial for understanding and predicting disease onset; however, reliable biomarkers for disease activity and progression are still lacking. Deploying powerful new technologies in the framework of collaborative networks to share biological samples from well-characterized and diverse patient cohorts is crucial. Research should also focus on unique populations with high disease prevalence or specific genotypes, and twin studies that can offer insights into the complex interplay and relative roles of genetics and environmental factors that affect the epigenome are key to understanding the disease [45, 46] . Leveraging interdisciplinary research, international collaboration, and standardized data collection will help identify new biomarkers. Integrating multi-omics data and refining preclinical models will advance SSc research and facilitate patient selection for clinical trials.

[H1] Novel technological breakthroughs and biomarker discovery

Clinical progress often results from technological breakthroughs; for example, the discovery of the patch clamp technology, the invention of cryo-electron microscopy, the development of state-of-the-art light microscopy, the advancement of genomic technologies and the improvements in computational methods. Technological advances have had a notable influence on SSc research. Innovations such as next-generation sequencing have accelerated analyses of genome sequence variation, of gene expression and of epigenetic markers. Next-generation sequencing has demonstrated that messenger RNA expression varies with clinical subsets of SSc, and with progression of the disease [11, 70-74]. Molecular classifications have been used to stratify patients in clinical trials such as for abatacept (as a post-hoc analysis) [75], and in HSCT. In many cases, it is clear that only a subset of patients responded to a specific treatment. Machine learning algorithms can use gene expression data for molecular classification in clinical trials, and gene expression could help predict outcomes, such as the modified Rodnan skin score (mRSS) [76-78]. As the volume of data increases, AI methods will become increasing important for data analysis.

Sequencing individual cell transcriptomes from biopsies has permitted detailed characterization of the cell types that are present, including fibroblasts, macrophages, lymphocytes, endothelial cells and keratinocytes. Advances in bioinformatics and computational methods have provided insights into spatial interactions between different cell types and provided a basis for hypotheses about their role in the disease [79-81]. The advent of spatial transcriptomics, which enables gene expression to be imaged at a single-cell resolution in histological sections has provided further insight into active gene programs and in interactions among different cell types involved in disease development [82-84]. Single-cell-resolved spatial protein analysis has also progressed, including techniques that can pinpoint activity states in cellular signalling cascades by reflecting dynamic protein interactions and post-translational modifications [85]. Automated detection of numerous proteins in situ, with advanced bioinformatics, enables comparison of data across labs, which is crucial for research in rare diseases [86]. Standardization of procedures and the development of novel in vitro systems, such as organoid models and tissue slices ,could enhance drug testing and understanding of SSc [87]. **[**

Methods have also become available that permit the levels of thousands of proteins to be measured in minute amounts of sample, such as blood plasma and tissue lysates. Available platforms are based on detection of specific blood proteins by DNA-aptamers [88], or using pairs of oligonucleotide-conjugated antibodies [89]. Thanks to the ongoing development of AI-based machine analyses, progress in the analysis protein expression patterns with diagnostic and prognostic value can be anticipated. The analyses can be applied to blood samples collected by donors themselves from a finger prick and sent by mail for storage in a dry state (90). This technology paves the way for convenient measurements of protein levels upon repeated sampling, sensitively reflecting disease processes Profiling of blood protein levels serves as a diagnostic tool in SSc [91] as has been reported for many forms of cancer [92]. Similar methods can also be used for comprehensive measurements of autoantibody repertoires in individual patients.

To exploit technological breakthroughs in SSc research it will be important to build biobanks with samples that are compatible with these emerging technologies. Specifically, fresh-frozen or formalin-fixed tissue sections are required to take advantage of spatial transcriptomics. In patients with ILD, meaningful information can be provided by lung tissue obtained via cryobiopsy, which serves as an alternative to surgical lung biopsy, when performed by experienced hands, with standardized protocols [93]. Similarly, blood samples, consecutively collected from large groups of individuals and inexpensively stored in a dry state, will enable monitoring of disease progress and responses to therapy. Such samples will also be crucial to the identification of blood biomarkers and other robust biomarkers, such as collagen-derived peptides, which indicative of ECM or degradation, the altered expression of which could be used to predict onset of disease Protein biomarkers will assume increasing importance if and when methods become available to avert disease onset Repeated blood sampling and molecular imaging (such as fibroblast-activation protein quantification with PET-CT) offers a way to monitor disease-relevant events. Combining these advances with systemic [research approaches will help identify new therapeutic targets and biomarkers, paving the way for improved clinical trials and treatment strategies (Figure 4).

[H1] Emerging therapies and translational research opportunities

Numerous novel treatment strategies with a wide variety of distinct mechanisms are being explored in clinical trials of SSc. The current pharmacologic and non-pharmacologic approaches, such as organ transplantation, and their clinical development phases have been reviewed elsewhere [94-96] . Thus far, no therapy that targets a single cell or molecule has induced long-term drug-free full remission of any autoimmune disease. Cell-based and targeted cellular depletion therapies are emerging as options for selectively modulating the immune response, mitigating vascular damage and the symptoms of Raynaud phenomenon, and also slowing or reducing fibrosis in skin and other organs , and promoting tissue repair (Table 2). Important developents include the use of CAR T cells, particularly CD19-targeting CAR T cells, which were shown to have clinical efficacy and relative safety in patients with SSc, along with other autoimmune diseases [97-99]. Although data are still limited, early evidence suggests that treatment with anti-CD19 CAR T cells might be better tolerated than autologous HSCT. This therapy might offer a more complete depletion of $CD19^+$ cells than B cell-depleting antibodies such as rituximab [100], potentially resetting the immune system. Despite the tremendous promise of this treatment and the surrounding great excitement, careful longitudinal studies are needed to confirm these findings, optimize the treatment protocols and patient selection criteria and explore the persistence of antibodies against other antigens Autologous HSCT, which 'resets' the immune system, has demonstrated substantial clinical benefit in SSc, particularly in improving skin manifestations, vascular changes and lung function [101-103]; however, HSCT carries a risk for adverse effects [104], but recent efforts have improved these shortcomings [105]. The use of mesenchymal stem cells and harnessing the immunomodulatory and anti-inflammatory properties of these cells have shown promise in early clinical

trials. Ongoing research is focused on optimizing delivery methods, optimal therapeutic range, frequency of administration and understanding long-term effects. Finally, randomized controlled trials are required to provide definitive evidence for the efficacy of MSC-based therapy and differences from HSCT [106,107]. Research into the use of induced pluripotent stem cells, which remains at the preclinical stage, focuses on the potential of iPSCs to regenerate damaged tissues and the development of patient-specific therapies with a lower risk of immune rejection Other cell-based therapies under investigation include modulation of regulatory T cells that can restore immune tolerance; preclinical studies and early clinical trials have shown promise for this therapeutic approach [108]. More recently , the role of regulatory B cells has been investigated in *in vivo* models of SSc to test the ability of these cells to modulate autoimmunity and fibrosis [109-110]. Several other immune cell types are under investigation, such as monocyte and macrophage subsets, for which direct cell reprogramming and metabolism (via CD38) and polarisation strategies to effect tissue repair have been the focus. Modulating the activity of both dendritic cells (and other antigen-presenting cells) and natural killer cells is also under investigation; these approaches focus on regulating the immune responses and harnessing cell cytotoxic potential to treat autoimmune disease [111].

Another promising area involves fibroblast-targeting therapies; in this setting the design of non-viral vectors for nucleic acid delivery represents a promising perspective. These approaches aim to selectively modulate disease-relevant pathways in fibroblasts [112], potentially minimizing adverse effects on other cell types. Cell-surface markers such as fibroblast-activated protein, are being investigated for drug targeting and liposomal carrier coating [113]. A particularly fast-moving area of research focuses on the identification and characterization of distinct fibroblast subpopulations. Such cellular heterogeneity, only recently uncovered with the advent of single-cell transcriptomics, indicates that not all fibroblasts in lesional tissue are the same, with some subpopulations being relevant to disease progression and associated with specific cell-surface markers. Cell-based therapies offer hope for more effective and targeted treatments for SSc, although further research is needed to establish their safety, efficacy, and long-term outcomes.

Recent advances in the development of in vitro models include precision-cut skin and lung slices, which, when combined with omics techniques and bioinformatic methods, enable the testing of new therapies and the personalization of treatments. These precision-cut skin slices retain cellular niches, but unlike ex vivo skin biopsies, this method ensures that the entire specimen receives adequate oxygen and nutrition supply via diffusion. Furthermore, multiple slices can be created from a single biopsy, enabling a direct comparison of different therapies across slices from the same sample. The changes in these skin slices in response to test compounds or therapies can be analyzed in an unbiased manner through the use of omic approaches. Precision-cut skin slices can be utilized as an ex vivo trial approach for human SSc skin, but they can also be used to select optimal treatments for individual patients based on molecular responses [114-116]. Promising preliminary observations indicate that the molecular response to currently used therapies in precision-cut skin slices faithfully predicts clinical responses. This approach could thus be used to guide treatment individualised treatment selection. 3D skin-like tissues provide another in vitro model to study SSc [117, 118]. These tissues are constructed using SSc cells and have been shown to recapitulate key features of SSc skin, including increased tissue thickness, stiffness, fibrotic , and immune pathway activation. These in vitro tissues enable cell–cell and cell–matrix interactions that are not captured in 2D culture and provide an alternative preclinical testing model for potential therapeutics.

[H1] Designing more successful clinical studies

As already noted, no approved disease-modifying treatment exists for SSc, partly owing to challenges in conducting effective clinical trials. The heterogeneous nature of SSc, the absence of reliable biomarkers for disease monitoring,lack of validated outcome measures and variable clinical course complicates clinical trials [119]. To overcome these obstacles, the SSc community has collaborated to propose innovative trial designs

and updated protocols that are based on emerging knowledge (Table 1); recent strategies have focused on cohort enrichment and outcome measure selection [119,129,130]. For example, the RISE-SSc trial targeted patients with dcSSc at high risk of skin fibrosis progression [121]. The trial did not meet its primary endpoint, as it showed no improvement in mRSS in the placebo group however, rapid mRSS improvement in some patients highlighted limitations in the approach [121]. The ASSET trial used a definition of 'active disease' for cohort enrichments; however, mRSS improvement was reported in both the active treatment and placebo groups, indicating that active disease alone was insufficient for cohort enrichment [120,131]. In the same study, gene expression profiling of biopsy-obtained skin samples and stratification by molecular subset revealed differences in mRSS progression and treatment response. One subgroup of patients, termed the ' inflammatory subset' , had strong activation of the CD28 co-stimulatory pathway targeted by the treatment [120] The focuSSced trial applied cohort enrichment based on active disease and inflammatory markers but did not meet its primary endpoint [35]. Nevertheless, this trial highlighted the effectiveness of tocilizumab in preventing the decline of forced vital capacity in patients with SSc-ILD, leading to its FDA approval for SSc-ILD [132] (Table 1).

Key observations are that early disease (duration <18 months) and mild skin thickening are useful for cohort enrichment [130]. Molecular measures of heterogeneity, such as gene expression in skin or blood can clearly be used as secondary endpoints as demonstrated in the ASSET clinical trial. Combining autoantibody information, such as excluding patients that are anti-centromere antibody-positive, can be informative. Disease alone is insufficient for cohort enrichment for those with skin progression. Combining biomarkers and omics data could improve cohort enrichment and treatment-response prediction. Inflammatory gene expression patterns in skin predict subsequent skin thickening and responses to certain treatments but this method is not yet viable for patient selection [72].

The RESOLVE-1 trial, which had minimal cohort enrichment, used the ACR-CRISS (ACR Composite Response Index in Systemic Sclerosis) , but the high score in the placebo group indicated that background immunosuppressive treatments might have influenced results [106]. The SENSCIS trial, by contrast, successfully demonstrated the efficacy of nintedanib in SSc-ILD, with a significant reduction in the decline of forced vital capacity over 52 weeks and enrolled a broad range of patients with SSc-ILD [8] (Table 1.). Challenges remain in using mRSS as a primary endpoint owing to its tendency to improve over time, necessitating cohort enrichment. Composite measures such as ACR-CRISS have shown varied results [133], and new endpoints such as revised CRISS-25 and wearable devices could offer future solutions [134]. In the future, the use of omics data to identify reliable biomarkers and rapid skin gene expression profiling will be essential for selecting patients likely to respond to a given therapy and for evaluating therapeutic efficacy. The platform clinical trial adopted by CONQUEST could be an alternative trial design that accommodates sample size reduction and robust patient participation [135]. In this setting , innovative designs such as digital twins connected devices, AI and mathematical modelling could be proposed to test or validate personalized therapeutic strategies in groups of patients stratified according to specific biomarkers. Patient-centred trial design that involves patient organizations is crucial for addressing real-world medical needs.

[H1] Future perspectives

Studying rare diseases such as SSc and developing therapeutic trials requires a specific approach. International cooperation on a global level has been successful in the collection of sufficient data from well-characterised cohorts of patients. The need for this approach to research has already been recognised by the scientific community, which is reflected in the many recent publications for several other disease entities, which list several specialised centres and research groups as co-authors.

There was, therefore, a clear consensus at this symposium that these developments need to be strengthened and brought from national and regional levels to a global level. The final aim should be to generate a common database that combines clinical investigations involving regular follow-up of patients with the molecular and cellular analysis of biopsy-obtained skin samples and blood samples. This approach will require complex

organization of multiple centres taking into consideration all legal and ethical aspects of data collection and transmission within and between networks on national, regional and intercontinental levels.

However, the rapid developments of methods of molecular and cellular analyses combined with computational methods, including AI and detailed clinical investigations, now offer a unique opportunity to better understand this complex, heterogeneous disease and to develop personalized therapeutic interventions. Determining a common definition of SSc and the development of a unified classification for all disease subtypes is essential. A consensus for common protocols for clinical trials (encompassing both inclusion criteria and treatment regimens) is required, and systematic cooperation with industrial partners early on will be mandatory. Biopsies and blood samples should be collected from all patients under standardized conditions and analyzed using state-of-the-art technologies, including single-cell RNA sequencing, proteomic and metabolomic approaches, to generate an atlas for SSc

There is also need for consensus regarding the selection of animal models and other cell and organoid-based in vitro models for testing novel hypotheses developed from basic research studies and for screening new compounds, which again needs to be achieved in cooperation with industrial partners.

Large data sets obtained from next-generation DNA sequencing techniques (including analysis of noncoding sequences, somatic mutations and methylation) need to be correlated with clinical, genetic and additional molecular data to identify very rare mutations in selected patients and their families as this information could have important implications for understanding SSc in general. Selecting populations and/or phenotypes that provide the largest signal-to- noise is critical to success. An excellent example is the GRASP study which has focused on African Americans with SSc who have a more severe disease and poorer outcomes Based on large cohorts of very well characterized patients these international consortia will rapidly be able to carry out specific trials and evaluate distinct currently unanswered questions. Examples of studies that are needed include studies that determine the value of short-period pilot studies, studies that evaluate the highly efficient elimination of B cells as a therapeutic advantage and studies that evaluate whether treatment of patients who meet the VEDOSS criteria for very early SSc VEDOSS might prevent further development of the disease . Precision medicine approaches that could enrich studies for populations that aremost likely to improve on specific treatments [could dramatically increase the success rate of trials and ensure that patients get the most effective treatment.

The discussion during the workshop demonstrated that the SSc research community has already provided several examples of how generating networks between centres can work effectively and patient cohorts have been generated in the USA, Europe, Canada, Australia and Japan. Several of these activities have initially been funded by national governments but often only for a short initial period. The participants of the workshop reached agreed that it is now time to join such individual activities to raise awareness for SSc in the medical community and the public to facilitate better and earlier diagnosis and treatment and to implement measures on different levels to secure funding . The cooperation of the scientific community with industrial partners and patient organizations should advance research on SSc even further for the benefit of the patients.

[H1] Conclusions

The integration of cutting-edge and emerging research techniques into SSc studies is poised to transform the understanding of this complex disease. Omic analyses including bulk and single-cell analyses, spatial transcriptomics, proteomics, epigenetics and comprehensive cell and protein atlases, coupled with computational analyses for pathways, cell types and genetic variations, offer unprecedented insights into the molecular and cellular underpinnings of SSc. As these technologies continue to evolve, they will have a crucial role in advancing precision medicine, identifying novel therapeutic targets, and ultimately improve the lives of patients with SSc. The future of SSc research is bright, with these innovative approaches paving the way for important breakthroughs in diagnosis, treatment and patient care.

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Author contributions

The authors contributed equally to all aspects of the article.

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Competing interests

J.H.W.D has consultancy relationships with AbbVie, Active Biotech, Anamar, ARXX, AstraZeneca, Bayer Pharma, Boehringer Ingelheim, Celgene, Galapagos, Genentech, GSK, Inventiva, Janssen, Novartis, Pfizer, Roche and UCB. and has received research funding from AbbVie, Anamar, Argenx, ARXX, BMS, Bayer Pharma, Boehringer Ingelheim, Cantargia, Celgene, CSL Behring, ExoTherapeutics, Galapagos, GSK, Inventiva, Kiniksa, Lassen, Novartis, Sanofi-Aventis, RedX, UCB and Zenasbio. J.H.W.D is CEO of 4D Science and Scientific Lead of FibroCure. R.D. Aisa Pharma Inc and AstraZeneca. M.K. Boehringer Ingelheim, Mochida, Kissei, GSK,

AstraZeneca, Mitsubishi Tanabe, Janssen, Biogen, Novartis, CHugai and Asahi Kasei Pharma. U.L. is the founder of Olink Proteomics, and stocks in Navinci and SampleFacts

Trial details	Drug	mRSS	mRSS change	FVC	CRISS	HAQ-DI	PtGA	PhGA
faSScinate[34] (n=86, 48 weeks)	Tocilizumab	0.06[b]	-3.5	0.03	0.002	0.53	0.51	0.03
focuSSced[35] (n=212, 48 weeks)	Tocilizumab	0.1[b]	-1.73	0.002	0.02	ns	ns	ns
ASSET[120](n=88, 52 weeks)	Abatacept	0.28	-1.75	0.11	0.03	0.005	0.73	0.03
RISE-SSc[121] (n=121, 52 weeks)	Riociguat	0.08	-2.34	ns	ns	ns	ns	ns
JBT-101-SSc[122] (n=38, 16 weeks)[a]	Lenabasum	0.085	-2.6	ns	0.04	0.03	0.1	0.02
RESOLVE-1[123](n=365, 52 weeks)[a]	Lenabasum	ns	ns	ns	ns	ns	ns	ns
FASST[124] (n=145, 48 weeks)[a]	Lanifibranor	ns	+0.9	ns	N/A	N/A	0.08	N/A
SENSCIS[8](n=580, 52 weeks)[a]	Nintedanib	0.58	-0.2	0.035	ns	N/A	N/A	N/A
Sanofi IL-4 and IL-13[125] (n=97, 24 weeks)[a]	Romilkimab	0.03	-2.31	0.10	ns	0.4	0.1	N/A
NOVESA[126] (n=33, 24 weeks)[a]	Ziritaxestat	0.04	-2.60	N/A	N/A	N/A	N/A	N/A
DESIRES[127] (n=56, 24 weeks)	Rituximab	0.001	-8.44	0.044	N/A	N/A	N/A	N/A
CERTA [128](n=30, 12 weeks)[a]	FT011	0.43	-1.5	0.018	0.05	0.019	0.94	0.02

For comparison between active and placebo arms at the end of each trial p-values are shown. For trials in which the primary endpoint was not met these are nominal values.
[a]Indicates that trial participants could have background immunosuppressive treatments
[b]Indicates that there was significant reduction in meaningful worsening of mRSS
ns, not statistically significant; N/A, data not available at time of writing; mRSS, modified Rodnan skin score; FVC, forced vital capacity; CRISS, Composite Response Index in Systemic Sclerosis; HAQ-DI, Health Assessment Questionnaire Disability Index; PtGA, patient global assessment of disease status; PhGA, physician global assessment of disease status.

Table 2 Approaches to cell-based therapies in SSc

Cells that could be targeted with cellular therapy	Approach to cell-based therapy
Monocytes	Cell ablation and polarisation
Macrophages	Cell polarisation
Fibroblasts	Pathogenic cell subset ablation
B_{REG} cells	Immune modulation (autoantibodies)
T_{REG} cells	Immune suppression (cytokines)
DCs	Cell ablation and cytokine production
B cells	Cell ablation

Commented [HW1]: Au: Is this baseline mRSS or final mRSS? Please also clarify this for the other outcomes.

Commented [HW2]: Au: Is this the ACR-CRISS or the CRISS-25 score?

NK cells	Modulate function
MSCs	Immune modulation and repair
iPSCs	Regeneration and repair
MDSCs	Cell-based therapeutics
HSCs	Cell replacement and immune reset
CAR T cells	Immune cell ablation

B_{REG} cells, regulatory B cells; T_{REG} cells, regulatory T cells; DCs, dendritic cells; NK cells, natural killer cells; MSCs, mesenchymal stem cells; iPSCs, induced pluripotent stem cells; MDSCs, myeloid-derived suppressor cells; HSCs, haematopoietic stem cells; CAR T cells, chimeric antigen receptor T cells.