

Scleroderma

Care and Research

Volume 1, Number 2
Winter 2003

Journal of the
Scleroderma Clinical
Trials Consortium



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Mission Statement

Scleroderma Care and Research is an independent, quarterly journal committed to elevating the standards of care in scleroderma and presenting new and useful information from ongoing clinical trials. It is the official journal of the Scleroderma Clinical Trials Consortium. The journal is distributed to rheumatologists in the United States and additional physicians internationally.

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Scleroderma Care and Research is circulated to the community of physicians caring for patients with scleroderma.

About the cover:

Digital ulcers in a patient with scleroderma recurrent in spite of selective digital sympathectomy/adventectomy.

Editor's Memo

Welcome to the second issue of *Scleroderma Care and Research*—the journal of the Scleroderma Clinical Trials Consortium (SCTC).

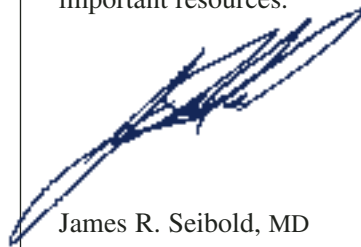
We are indeed in a historically vigorous period of basic, translational, and interventional research in scleroderma. While we don't yet fully understand the pathogenesis of scleroderma, we do feel confident that we know enough about the pathobiology of the disease to attempt truly rational intervention. Improved scientific understanding coupled with remarkable collaborative gains in measurement of the clinical phenomenology of this complex heterogeneous disorder has led to a veritable flood of well-designed and robustly powered randomized controlled trials.

Members of the SCTC include virtually all of your colleagues who focus their research and care on scleroderma. This journal offers us the opportunity to relay new knowledge and cutting-edge strategies to the broader community of caregivers.

This issue focuses on two diverse yet important areas. Our understanding of the complex genetic background associated with scleroderma is becoming increasingly refined. Assassi and Mayes provide a comprehensive review of the knowledge to date, along with instructive information for the clinician about techniques of genetic study. Denton and Korn tackle the difficult clinical problem of digital ulcerations. This is particularly appropriate for the winter months but also serves to highlight an ambitious SCTC-based study of the role of endothelin receptor antagonism.

It is our express intention to provide you with material that is useful in your practice. Protocol participation is the treatment of choice for many patients and we continue to urge that you consider SCTC trials as an approach to patient management. Visit our Web page (<http://www.sctc-online.org>) for updated information about trials and how to contact participating centers, and review the Current Studies section of this journal. The options are ever-changing.

Scleroderma Care and Research would not be possible without our partners at the Scleroderma Foundation, the International Scleroderma Network, and the Scleroderma Research Foundation. Information about each organization appears in these pages, and all have useful Web sites. Please alert your patients to these important resources.



James R. Seibold, MD
Editor-in-Chief

The Genetics of Scleroderma: What Every Rheumatologist Should Know

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There is increasing evidence of a genetic basis of systemic sclerosis (SSc, scleroderma). This article reviews the current data and provides an overview of the candidate genes that may influence disease susceptibility or severity.

Does Scleroderma Run in Families? Evidence for Familial Aggregation

The evidence that SSc has a familial component comes principally from two studies that examined large cohorts of patients.^{1,2} That this observation has only recently been made is likely due to the fact that SSc is a fairly uncommon disease (affecting approximately 1 in 4000 US adults). Familial recurrence occurs in only about 1.6% of families.³ To look at it from the opposite perspective, 98.4% of cases will *not* have another first degree family member affected with SSc. However, this recurrence frequency is greater than would be expected for the general population, resulting in an estimated relative risk of 13 fold of SSc among first degree family members.¹

These figures may seem counterintuitive. How can the *absolute* risk of a disease be so low (1.6% of families), while the *relative* risk for these family members is so high (13 fold)? The answer lies in the rarity of the disease and in the calculation of relative risk. The relative risk is a ratio of the prevalence observed in the study population (in this case, first degree family members) compared with the expected prevalence in the general population. This nuance may get overlooked in the interpretation of risk in discussions with patients who worry about passing the “scleroderma gene” on to their children.

Additional support for the role of genetic susceptibility factors comes from studies of Choctaw Native Americans in Oklahoma, a group with the highest reported prevalence of SSc to date, approximately 1/1500.⁴ In addition to this high disease frequency, disease expression in the Choctaw is relatively uniform, with

most cases having diffuse skin involvement, pulmonary fibrosis and positive antitopoisomerase antibodies (anti-Scl-70). Unique features of the Choctaw include descent from a small number of “founders” and relative genetic isolation. Subsequent genetic analysis, including a genome wide scan,⁵ suggested several gene regions contributing to this disease susceptibility, including HLA loci, fibrillin, and others (see below).

The finding of familial aggregation or of disease clustering in a comparatively closed population, implies, but does not establish, a genetic basis. The possibility remains distinct that a shared environmental factor may be responsible for this occurrence pattern.

Studies of Scleroderma in Twins

Twin studies are commonly utilized to distinguish genetic from environmental factors, since disease concordance among monozygotic (MZ) twins should be greater than among dizygotic (DZ) twins for diseases with a strong genetic component. Performing a twin study in a rare disease is a challenging task and only one study has been published to date. Feghali-Bostwick et al,⁶ reported that there was no difference in disease concordance between MZ and DZ twins (4.7% overall concordance for both twin types). However, concordance for the presence of antinuclear antibodies was significantly higher in the MZ twins compared with their DZ counterparts. This intriguing finding seems to place scleroderma partway between a genetically determined disease and an environmentally acquired one.

These studies taken together suggest that while there are genetic features that contribute to disease development, there are also additional and essential acquired factors that must be present in order for disease to occur. The acquired factor is presumably one or more environmental trigger. Considering that the onset of SSc is usually in midlife (average age of onset is 45 years), it may be that only one member of the twin pair experiences the event or events and goes on to develop disease.

The low *absolute* risk of disease for first degree family members also argues in favor of an interplay between genetics and the environment. It is likely that multiple genetic factors, each contributing a modest effect, combine with multiple environmental triggers to initiate disease. This multiplicity of factors is the definition of a “complex” genetic disease and distinguishes these conditions from the monogenic or one gene mutation leading to one disease model. Such complex diseases show heritability demon-

strated by familial aggregation but do not follow any known mode of inheritance, due to the requirement of interaction among multiple genes and multiple potential triggers.

This complex genetic model also offers a potential explanation for the observed heterogeneity of scleroderma in disease expression, both clinically and serologically. This has implications for both prognosis and treatment. If there are various pathways to disease development and/or progression in different patients, then blocking one mechanism may not help all subjects. Ultimately, the ability to subset patients on the basis of genetic predisposition may influence treatment decisions.

Mechanisms of Genetic Variation: Gene Duplications and Deletions

The tight skin (TSK-1) murine model of scleroderma is an example of a gene duplication causing a disease phenotype.⁷ The duplication results in the production of a fibrillin protein that is larger than normal. Although the mechanistic link between the abnormal fibrillin production and the subsequent dermal fibrosis has not been precisely determined, it is thought that increased release of transforming growth factor beta (TGF- β) from its normal binding site on fibrillin contributes to the excess collagen production.

A gene duplication event is a “major” genetic mutation and has not been found in human scleroderma. In the TSK-1 mice, homozygotes die in utero and only heterozygotes survive to demonstrate the phenotype. However, this model served to identify fibrillin as a potentially important regulator of collagen matrix production and alterations in this pathway are being investigated in the human disease.

Mechanisms of Genetic Variations: Polymorphisms

It is likely that genetic polymorphisms, multiple normal variants of common genes, rather than gene deletions or duplications are responsible for increased disease susceptibility in scleroderma and other autoimmune diseases. Such polymorphisms may cause no change in the function of the gene product, or only a relatively small change in function that becomes biologically important only under particular circumstances. As a further complicating factor, these alleles need not necessarily be in the translated regions that directly affect a protein product, but may be in promoter regions, in binding areas for transcription factors, or may involve mechanisms that influence post-translational modifications.

Although the human genome project has successfully identified many genes and gene regions, the identification of the all of the normal variants of these genes (common polymorphisms) has only begun. It has been estimated that there may be only 35,000 genes in the human genome, but *several million* polymorphisms. A typical example is the ABO blood group system, in which the gene coding for this red cell surface protein has three major alleles: A, B, and O. The genes are codominant, which is to say that an individual who has inherited the allele coding for the A protein from one parent and the B allele from the other parent will express both surface proteins and will be blood type AB. The frequencies of particular alleles will vary among populations that are geographically (ethnically) diverse.

A common method to study the role of genetic polymorphisms

in disease is to use a candidate gene approach in which a relevant gene with several known polymorphisms is chosen and the frequencies of particular polymorphisms are compared between patients and controls.

Transmission-Disequilibrium Testing

Transmission-disequilibrium testing (TDT) is an analytical approach that uses a parent-offspring trio design to identify associations between particular alleles and disease status. Candidate genotypes for the unaffected parents are determined (each parent having two alleles for the candidate gene) and the genotype of the affected offspring is determined. This is repeated for multiple families and the frequency of transmission to the offspring of the putative disease allele is calculated. If the candidate allele is not related to disease, there will be no “excess” transmission to affected individuals. By using family members as unaffected controls, this method largely eliminates the problem of variation among ethnic groups in allele frequency. In some situations in which only one parent is available for study, one or more healthy siblings can be substituted for the missing parent.

Microsatellite markers are typically used to screen relatively large numbers of cases and controls. Particular markers are chosen that are in linkage disequilibrium with the region of the candidate gene (presumed disease allele). Linkage disequilibrium implies that the marker is so “close” to the disease allele that both will be transmitted together. Use of such markers is technically easier than identification of individual gene polymorphisms and does not require that all polymorphisms of the candidate gene be known. On the other hand, this approach identifies candidate gene *regions*, large areas containing multiple genes including the candidate gene of interest. Once the gene regions are identified, additional studies need to be done to determine which specific allele is responsible for the association.

Genome-Wide Studies

The candidate gene approach requires the selection of a likely polymorphism and then tests the case and control populations for the frequency of the particular variant. A genome-wide scan uses a different approach, comparing variations between the case and control populations over the entire genome in order to identify any and all gene regions that distinguish the disease group from the control group. Again, once identified, these gene regions must be further analyzed to determine which particular alleles are associated with the disease phenotype. The value of the genome-wide scan is that no assumptions need to be made ahead of time about which genes are important. This approach can usually be applied only to multicase families or to relatively closed populations in order to diminish the expected differences among individuals in an outbred population.

The first application of a genome-wide association study in scleroderma was conducted in Oklahoma Choctaw Native Americans. The **Figure** shows the ancestral origins of 20 contemporary Choctaw SSc patients tracing these cases to five founding families in the late 1700s, suggesting that common founders may have introduced disease genes into the Choctaw population about 10 generations ago.⁸

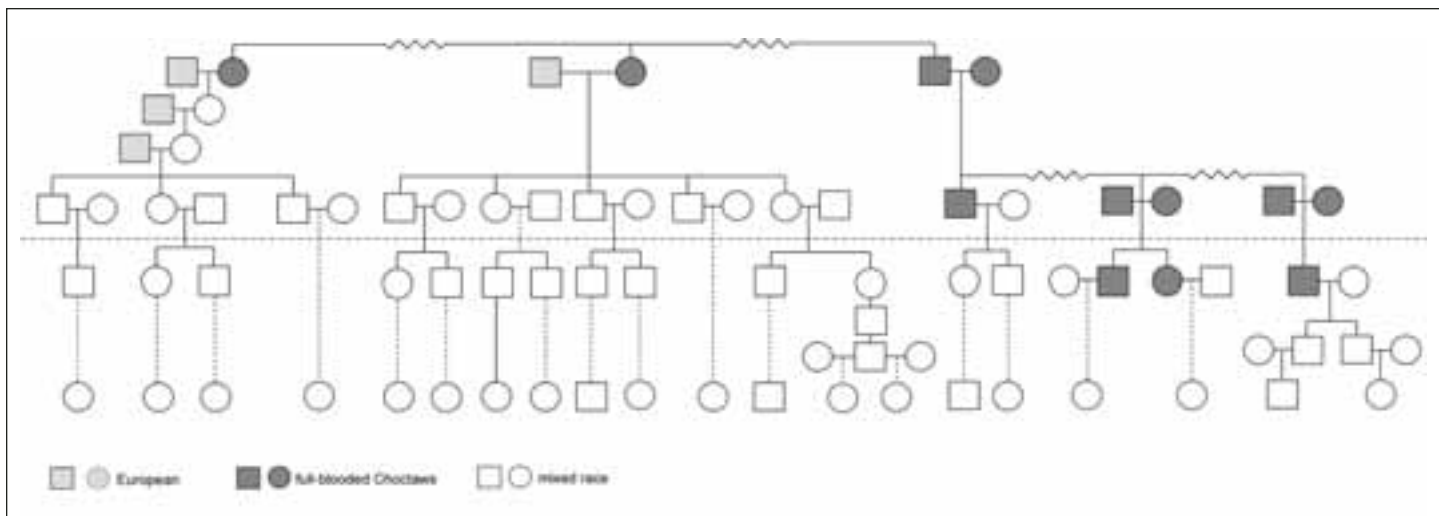


Figure. Ancestral origins of 20 Choctaw SSc patients. Scleroderma cases are the individuals arranged along the lower row. Single generations are shown by solid lines and multiple generations by broken lines. Hypothetical links between family clusters are indicated by the jagged lines. These links were established by circumstantial archive evidence. The individuals above the broken horizontal line indicate those born before the removal to Indian Territory in 1831-1859. Reprinted by permission from the publisher (see reference 8).

In the Choctaw study, a genome-wide microsatellite screen at low resolution (10cM resolution using 400 markers) was performed in 20 patients and 76 ethnically matched controls.⁵ Based on the initial results, a fine-scale microsatellite mapping at a higher resolution (<1 cM) was conducted in 10 selected chromosome regions. From the original 400 investigated markers, 12 showed significant association with the disease, including markers near the Fibrillin-1 (*FBNI*), *SPARC* (Secreted Protein, Acidic and Rich in Cysteine) and *TOPO1* (topoisomerase 1) genes. A correlation with loci previously reported to be linked to SLE and other autoimmune diseases was also detected. In addition to these ‘autoimmune’ regions, eight markers were novel regions found to be uniquely associated with scleroderma. These results suggest that multiple genetic loci contribute to the high prevalence of SSc in this population and reflect the fact that some autoimmune rheumatic diseases are likely to share common genetic determinants. The precise nature of the genes involved and their role in pathogenesis is not yet clear.

Candidate Genes

There is no shortage of candidate genes to study. In scleroderma, the usual suspects include those of the HLA region that influence immune responses, inflammatory mediators, particularly TGF- β , which has been shown in multiple studies to occupy a pivotal role in SSc pathogenesis,⁹ matrix components, and vascular factors. In addition to genes coding for these protein products, candidates also include the promoters, transcription factors, and cell surface receptors that mediate the effects of these compounds. The following is a summary of these findings.

Candidate Genes in Family-Based TDT Studies

The technique of transmission-disequilibrium testing was used to study several candidate gene regions in a group of SSc patients and their first degree relatives.¹⁰ In this study, 60 pedigrees con-

taining 61 SSc patients with either both parents available (29 families) or one parent and at least one or more siblings (31 families) were studied. Several microsatellite markers and their alleles showed preferential transmission to the affected individuals. Candidate genes within these regions included fibrillin 1 (*FBNI*) (15q21), platelet derived growth factor (22q13), *SPARC* (5q31.3-5q32), the SLE linked region 1q42, which also contains the estrogen-related receptor gamma gene (*ESRRG*), and the MHC (6p21). This study is being extended to additional families with more precise techniques to identify these areas.

HLA Associations

HLA-DR, DQ, and DP molecules are expressed as α : β heterodimers on the surface of B cells, dendritic cells, activated T cells, and other somatic cells and are required for recognition of foreign (exogenous) antigens by T helper cells. The genes encoding the α and β chains reside in the class II region of the MHC. The first report of an association of SSc in some individuals with certain HLA alleles was made nearly 25 years ago.¹¹ Since then multiple associations have been reported in different ethnic groups. The strongest correlations have been seen with particular autoantibody responses such as antitopoisomerase 1 antibody production and *DRB1*1101*-*1104; *DQA1*0501*; *DQB1*0301*, and *DPB1*1301* in Caucasians and African-Americans. Other HLA haplotypes have been associated with anticentromere antibodies and these associations differ depending on ethnicity.

If there is so much variation by ethnicity, how can these associations be relevant? One interpretation of these data is that the various “ethnic” alleles code for cell surface proteins that share common antigen recognition sites. According to this interpretation, for example, a “Japanese” HLA type that appears different from a “European” HLA type will actually code for a cell surface protein that recognizes similar antigens, leading to a similar immune response. This interpretation will remain speculative until

the putative antigens are identified and the hypothesis tested. However, since SSc disease severity correlates with autoantibody type, and HLA associations correlate with autoantibody production, it seems likely that HLA links are an important piece of the puzzle.

Transforming Growth Factor Beta

Transforming growth factor beta (TGF- β) is a cytokine that stimulates production of various matrix proteins including collagen and fibronectin through activation of the Smad pathway.^{9,12} Inhibition of the Smad pathway has resulted in inhibition of skin fibrosis in the tight skin mouse model of human SSc.¹¹ In addition, immunohistochemical studies have shown increased TGF- β levels in the extracellular matrix of affected SSc skin.¹³

A single nucleotide polymorphism (SNP) of the TGF- β (+869 C \rightarrow T) gene has been investigated in SSc. The C allele (cytosine substitution for thymine at position 869) correlates with higher levels of TGF- β . Two studies^{14,15} have shown that the C allele was significantly more common in SSc patients compared with controls, occurring in 48% of SSc patients compared with 38% of controls in the UK study;¹⁴ and the C allele occurring in 65.3% of the cases versus 50.5% of the healthy controls in the Japanese study.¹⁵ It is noteworthy that this finding was similar in two different ethnic groups.

In contrast, a study of this TGF- β polymorphism in the Choctaw population¹⁶ did not find an association with disease. This may be a reflection of true differences in susceptibility gene-sets between these ethnically different groups, or the lack of confirmation in the Native American group may mean that this is not a disease-relevant polymorphism. These differing findings demonstrate that there is as yet no firm consensus regarding the significance of TGF- β alleles in SSc.

Tumor Necrosis Factor

Tumor necrosis factor (TNF) is a proinflammatory cytokine. In SSc patients, elevated levels of TNF α and soluble TNF receptors have been reported in the serum, skin, and bronchoalveolar lavage fluid,^{17,18} supporting the nomination of TNF as a candidate gene.

One study investigated a TNF β ⁺²⁵² microsatellite in Japanese SSc patients and found that homozygosity for the TNF β ⁺²⁵² allele, TNFB*2, was significantly associated with diffuse scleroderma.¹⁹ Another study determined TNF α and TNF β microsatellite polymorphisms in Japanese and German SSc patients and in normal controls. No association was found among the German patients who had SSc, but the frequency of TNF α 13 was significantly increased in the Japanese SSc patients.²⁰ However, it is not clear if these microsatellites influence TNF functional activity.

Interleukin-1A

Interleukin-1A (IL-1A) activates IL-6 and platelet-derived growth factor (PDGF-A), which can induce collagen production and proliferation. Constitutive expression of IL-1A has been observed in SSc but not in normal fibroblasts.²¹

A case-control study investigated three single nucleotide polymorphisms (SNPs) and inferred haplotypes in SSc patients and healthy controls.²² In this study, the CTG haplotype was signifi-

cantly associated with the disease and with interstitial lung involvement. This study also suggested that pharmacological interventions aimed at IL-1A blockade might be beneficial in SSc. Since the majority of constitutively expressed IL-1A is intracellular, traditional approaches to cytokine blockade may be ineffective. Short interfering RNAs that have a gene silencing effect are seen as a promising therapeutic approach.

Interleukin-4

Increased levels of IL-4 have been found in dermis, serum, and bronchoalveolar lavage/lung biopsy specimens of SSc patients,²³⁻²⁵ placing this gene on the candidate list. Again, the TSK mouse model has provided instructive data. IL-4 seems to be instrumental in development of cutaneous fibrosis in the TSK mouse²⁶ and disruption of the IL-4 gene decreases the production of TGF- β by fibroblasts and rescues TSK homozygotes from in utero death.²⁷ In terms of human disease, a variant in the IL-4 receptor region was found to be associated with SSc, Sjogren's syndrome, and SLE.²⁸

Fibrillin 1

Fibrillin 1 is a glycoprotein that is the principal constituent of extracellular microfibrils. Mutations in the fibrillin 1 gene (*FBNI*) cause Marfan syndrome in which fibrillin production is diminished or lacking. A mutation in the fibrillin 1 gene results in the phenotype of the TSK-1 mouse, making this gene a prime SSc candidate. Microsatellites in and around the *FBNI* as well as *FBNI* SNPs on chromosome 15q21 were found to be associated with SSc in the relatively homogenous populations of Choctaw Native Americans and Japanese,^{28,29} although a defect in the *FBNI* gene itself has not yet been identified. Microfibrils in the skin of SSc patients,³⁰ and microfibrils from cultured SSc fibroblasts are abnormal at the ultrastructural level by electron microscopy³¹ and appear to be more readily degraded than those derived from normal controls.³² The cause of the underlying microfibril instability has not yet been identified.

Collagen

Increased levels of type I collagen have been reported in the extracellular matrix (ECM) of SSc patients. Type I collagen is encoded by two genes (*COL1A1* and *COL1A2*). Numerous studies have shown transcriptional activation of collagen genes in skin and lung fibroblasts of scleroderma patients.³³ Ubiquitous transcriptional factors like CCAAT binding factor (CBF) and Sp-1 play an important role in regulating the transcription of the collagen genes. The transcriptional activation in SSc fibroblasts seems to be partially secondary to higher levels of CBF³⁴ and Sp-1 phosphorylation.^{35,36}

In addition, alteration in ECM protein turnover could result in higher collagen levels, and altered levels of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMP), proteins that regulate the turnover of ECM, have been reported in SSc sera and skin.³³ However, a case-control study of a functionally relevant single nucleotide polymorphism of matrix metalloproteinase-1 promoter in a multiethnic US cohort showed no association with SSc susceptibility or with any of the major

clinical manifestations of SSc.³⁷ Another study of SSc in an Italian cohort did report an association between a promoter polymorphism in the MMP-3 gene and SSc,³⁸ but there was a significant deviation from Hardy-Weinberg equilibrium in the SSc group, raising concerns that this could be a chance finding.

Fibronectin

Fibronectin is another ECM protein that serves as a cell adhesion molecule by anchoring cells to collagen or proteoglycan substrates. In addition, fibronectin acts as a chemotactic substance to attract fibroblasts to areas of inflammation in the pulmonary tissue of SSc patients.³⁹ A marked increase in fibronectin has been found in the deep dermis of involved scleroderma skin, and the distribution of the accumulated fibronectin appeared to parallel that of the accumulated collagen in the involved reticular dermis.⁴⁰ Comparable to collagen, there seems to be transcriptional activation of the fibronectin gene in SSc fibroblasts.⁴¹ A case control study reported that certain restriction fragment length polymorphisms in the fibronectin gene were found more frequently in patients who had SSc and pulmonary fibrosis.⁴²

SPARC: Secreted Protein, Acidic and Rich in Cysteine

Secreted protein, acidic and rich in cysteine (SPARC) is an important regulator of extracellular matrix metabolism. Three SNPs in the *SPARC* gene were found to be associated with either disease susceptibility or disease expression in a multiethnic SSc cohort.⁴³ In addition, microsatellite analysis of the *SPARC* gene revealed 3 of 5 markers to be significantly associated with SSc in Choctaw Native Americans.⁴³ Homozygosity for the C allele was associated with SSc in all ethnic groups. This allele also correlated with longer mRNA half-life, suggesting that this mutation may be functionally important in influencing SPARC expression in fibroblasts.

eNOS and ACE

Nitric oxide synthase (NOS) is responsible for the production of nitric oxide, a potent vasodilator. Endothelial cells constitutively express endothelial NOS (eNOS) in the normal state. Inducible NOS (iNOS) is produced during inflammatory states, which leads to cellular injury by increased production of free oxygen radicals. Immunohistochemical studies on skin biopsies of SSc patients have shown eNOS expression was inversely related to the grade of skin disease, whereas iNOS staining was increased with higher levels of skin involvement.⁴⁴ These data suggest there is a shift from eNOS to iNOS in SSc patients.

Another relevant vascular pathway in SSc is the angiotensin system. Angiotensin I-converting enzyme (ACE) catalyzes the conversion of angiotensin I into the vasoactive and aldosterone-stimulating peptide angiotensin II, and inactivates bradykinin, a vasodilator. The *ACE I/D* polymorphism accounts for some of the observed variance of serum ACE levels in the general population, with the *ACE D* allele correlating with higher ACE levels. A study using DNA extracted from paraffin-embedded renal biopsy material from 48 patients with malignant vascular injury, including 10 with SSc, reported increased frequency of *ACE D* alleles compared with a panel of healthy controls.⁴⁵ A separate case-control

study reported significant association between *ACE D* polymorphism and *eNOS 894T* alleles and SSc.⁴⁶ However, a similar study in a large multiethnic population in Texas failed to show any correlation between the above-mentioned alleles and SSc.⁴⁷

Bone Morphogenetic Protein Receptor II

The identification of mutations in the bone morphogenetic protein receptor II gene (*BMP2*) as the genetic abnormality responsible for most cases of familial primary pulmonary hypertension (PPH), as well as some sporadic cases, is one of the most significant genetic discoveries in recent years.⁴⁸ *BMP2* is a member of the TGF- β receptor superfamily and multiple types of mutations in the kinase portion of this gene have been reported, including missense, nonsense, and frameshift mutations. Although the precise mechanism leading to PPH is not clear, it is proposed that the mutations interrupt normal receptor signalling which then results in proliferation, rather than apoptosis, of cells within small arterioles. Since the histological features of pulmonary artery hypertension (PAH) in the setting of scleroderma is undistinguishable from PPH alone,⁴⁹ the *BMP2* gene became a prime candidate for SSc-related PAH. However, two separate studies failed to find any *BMP2* mutations in these patients,^{50,51} suggesting that genetic defects other than *BMP2* might be involved in the pathogenesis of PAH in this disease.

Summary

Although much has been learned about the genetics of scleroderma, this review illustrates that much more needs to be done. The unique Choctaw population has provided remarkable insight into potential genetic links. Some of these links have been confirmed in studies of different ethnic groups while others are reported in one group but not found in all. This should come as no surprise. One can speculate that there are several scleroderma genotypes or combinations of multiple genes that comprise several distinct scleroderma genetic profiles. Should we be simultaneously measuring multiple polymorphisms in the same patients? Should we expand our single-case family studies? Should we be studying those few but important multicase families? These authors would answer yes to these questions.

In order to further this endeavor, the Scleroderma Family Registry and DNA Repository was established by the NIH/NIAMS to study disease-associated genes and to establish a repository of DNA, sera, and plasma from SSc cases, first degree family members, and spouse/friend controls to expedite genetic research. De-identified clinical and demographic data are also available. As of this writing, the repository contains samples from more than 350 verified cases, 1000 family members, and 200 unrelated controls. Information about access to the repository samples and database can be found at www.sclerodermaregistry.com. Applications are encouraged.

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Digital Ulceration and Critical Digital Ischemia in Scleroderma

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Digital ulcerations are a major clinical problem in scleroderma, occurring in about one third of patients per year. Digital ulcerations occur in both *diffuse* scleroderma, in which there is skin sclerosis involving the chest and abdominal wall and limbs proximal to the elbows or knees, and in *limited* scleroderma, in which skin involvement is restricted to the distal limbs. The vascular manifestations of the two major scleroderma subsets are listed in **Table 1**.

Digital ulcers may be present on the fingertips, in the finger creases, over extensor surfaces of joints, and in association with underlying calcinosis. Once present, ulcers cause local pain and functional impairment. Recent studies suggest that digital ulcer burden has a major negative impact on quality of life for scleroderma patients. Several pathological mechanisms operate in scleroderma to cause digital ulceration and critical ischemia. The latter describes the context in which there is inadequate tissue nutrition affecting a digit but without ulceration. It is frequently painful and is generally taken as a sign of impending ischemic ulceration. Local trauma, such as cuts or abrasions, may be an initiating event that, in concert with impaired healing because of poor vascular

flow, dermal fibrosis, and epidermal atrophy, may lead to chronic ulcerations. The factors underlying ulceration and critical ischemia are reviewed below.

Raynaud's Phenomenon

Raynaud's phenomenon is present in almost all patients with scleroderma, although the severity of symptoms varies greatly. In general, cold-induced vasospasm precedes the development of other features of the disease in the limited cutaneous subset of scleroderma, previously termed the CREST syndrome (for calcinosis, Raynaud's phenomenon, esophageal swallowing difficulty, sclerodactyly, and telangiectasia), often by many years. In contrast the onset of vascular symptoms is often more contemporaneous with other manifestations in patients with diffuse cutaneous systemic sclerosis. Persistent vasospasm may lead to impaired tissue oxygenation and development of digital ulcers. A range of vasodilator and other agents has been identified as potentially beneficial for Raynaud's phenomenon (**Table 2**), although Raynaud's secondary to scleroderma is generally more resistant to vasodilator treatment than the primary phenomenon.

Structural Vasculopathy

Although the vascular lesion in scleroderma is initially functional, ie, vasospastic, with time most patients develop progressive structural vascular disease. Biopsy and tissue studies of digits suggest that there is a fibroproliferative lesion in the digital arteries of patients with scleroderma that can lead to substantial narrowing of the lumen. This may be akin the changes that occur in other vascular beds including the pulmonary arterial tree in isolated pul-

monary arterial hypertension and in the interlobular arteries and cortical blood vessels of patients developing hypertensive scleroderma renal crisis. Marked pathological similarities have been observed in amputation specimens (**Figure**). It is likely that these changes also occur more proximally, and there are now many cases in which occlusive arteriopathy, especially in the ulnar artery, has been reported. It is unlikely that such structural changes can easily be influenced by vasodilator therapy.

Thrombosis

The role of intraluminal thrombosis

Table 1—Vascular Disease in Scleroderma

	Diffuse Scleroderma	Limited Scleroderma*
Frequency	30% to 40%	60% to 65%
Skin involvement	Proximal trunk and extremities	Fingers, feet, face
Raynaud's phenomenon	Concomitant with disease onset	May precede other features by years
Telangiectasia	May be present	Often extensive, florid
Renal disease	30% to 40%	None
Pulmonary fibrosis	30% to 40%	10% to 20%
Pulmonary hypertension	20%, usually with interstitial lung disease	Late, 20% to 50%
Digital ulcers	Common	Common

*This limited cutaneous subset of scleroderma was previously termed CREST syndrome (see text).

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WHO CLASS III OR IV**

- From <10%¹ to 50%² of scleroderma patients develop PAH
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Liver and pregnancy warnings

- Requires attention to two significant concerns
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 - High potential for major birth defects: Pregnancy must be excluded and prevented by two forms of birth control; monthly pregnancy tests should be obtained
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*Clinical worsening defined as combined endpoint of death, hospitalization or discontinuation due to worsening PAH, or initiation of epoprostenol therapy⁶

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Use of TRACLEER® requires attention to two significant concerns: 1) potential for serious liver injury, and 2) potential damage to a fetus.

WARNING: Potential liver injury. TRACLEER® causes at least 3-fold (upper limit of normal; ULN) elevation of liver aminotransferases (ALT and AST) in about 11% of patients, accompanied by elevated bilirubin in a small number of cases. Because these changes are a marker for potential serious liver injury, serum aminotransferase levels must be measured prior to initiation of treatment and then monthly (see WARNINGS: Potential Liver Injury and DOSAGE AND ADMINISTRATION). To date, in a setting of close monitoring, elevations have been reversible, within a few days to 9 weeks, either spontaneously or after dose reduction or discontinuation, and without sequelae. Elevations in aminotransferases require close attention (see DOSAGE AND ADMINISTRATION). TRACLEER® should generally be avoided in patients with elevated aminotransferases (> 3 x ULN) at baseline because monitoring liver injury may be more difficult. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin ≥ 2 x ULN, treatment should be stopped. There is no experience with the re-introduction of TRACLEER® in these circumstances.

CONTRAINDICATION: Pregnancy. TRACLEER® (bosentan) is very likely to produce major birth defects if used by pregnant women, as this effect has been seen consistently when it is administered to animals (see CONTRAINDICATIONS). Therefore, pregnancy must be excluded before the start of treatment with TRACLEER® and prevented thereafter by the use of a reliable method of contraception. Hormonal contraceptives, including oral, injectable and implantable contraceptives should not be used as the sole means of contraception because these may not be effective in patients receiving TRACLEER® (see Precautions: Drug Interactions). Monthly pregnancy tests should be obtained.

Because of potential liver injury and in an effort to make the chance of fetal exposure to TRACLEER® (bosentan) as small as possible, TRACLEER® may be prescribed only through the TRACLEER® Access Program by calling 1 866 228 3546. Adverse events can also be reported directly via this number.

INDICATIONS AND USAGE: TRACLEER® is indicated for the treatment of pulmonary arterial hypertension in patients with WHO Class III or IV symptoms, to improve exercise ability and decrease the rate of clinical worsening.

CONTRAINDICATIONS: TRACLEER® is contraindicated in pregnancy, with concomitant use of cyclosporine A, with co-administration of glyburide, and in patients who are hypersensitive to bosentan or any component of the medication.

Pregnancy Category X. TRACLEER® is expected to cause fetal harm if administered to pregnant women. The similarity of malformations induced by bosentan and those observed in endothelin-1 knockout mice and in animals treated with other endothelin receptor antagonists indicates that teratogenicity is a class effect of these drugs. There are no data on the use of TRACLEER® in pregnant women. TRACLEER® should be started only in patients known not to be pregnant. For female patients of childbearing potential, a prescription for TRACLEER® should not be issued by the prescriber unless the patient assures the prescriber that she is not sexually active or provides negative results from a urine or serum pregnancy test performed during the first 5 days of a normal menstrual period and at least 11 days after the last unprotected act of sexual intercourse. Follow-up urine or serum pregnancy tests should be obtained monthly in women of childbearing potential taking TRACLEER®. The patient must be advised that if there is any delay in onset of menses or any other reason to suspect pregnancy, she must notify the physician immediately for pregnancy testing. If the pregnancy test is positive, the physician and patient must discuss the risk to the pregnancy and to the fetus.

WARNINGS: Potential Liver Injury: Elevations in ALT or AST by more than 3 x ULN were observed in 11% of bosentan-treated patients (N = 658) compared to 2% of placebo-treated patients (N = 280). The combination of hepatocellular injury (increases in aminotransferases of > 3 x ULN) and increases in total bilirubin (≥ 3 x ULN) is a marker for potential serious liver injury. Elevations of AST and/or ALT associated with bosentan are dose-dependent, occur both early and late in treatment, usually progress slowly, are typically asymptomatic, and to date have been reversible after treatment interruption or cessation. These aminotransferase elevations may reverse spontaneously while continuing treatment with TRACLEER®. Liver aminotransferase levels must be measured prior to initiation of treatment and then monthly. If elevated aminotransferase levels are seen, changes in monitoring and treatment must be initiated. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin ≥ 2 x ULN, treatment should be stopped. There is no experience with the re-introduction of TRACLEER® in these circumstances. *Pre-existing Liver Impairment:* TRACLEER® should generally be avoided in patients with moderate or severe liver impairment. In addition, TRACLEER® should generally be avoided in patients with elevated aminotransferases (> 3 x ULN) because monitoring liver injury in these patients may be more difficult.

PRECAUTIONS: Hematologic Changes: Treatment with TRACLEER® caused a dose-related decrease in hemoglobin and hematocrit. The overall mean decrease in hemoglobin concentration for bosentan-treated patients was 0.9 g/dl (change to end of treatment). Most of this decrease of hemoglobin concentration was detected during the first few weeks of bosentan treatment and hemoglobin levels stabilized by 4-12 weeks of bosentan treatment. During the course of treatment the hemoglobin concentration remained within normal limits in 68% of bosentan-treated patients compared to 76% of placebo patients. The explanation for the change in hemoglobin is not known, but it does not appear to be hemorrhage or hemolysis. It is recommended that hemoglobin concentrations be checked after 1 and 3 months, and every 3 months thereafter. If a marked decrease in hemoglobin concentration occurs, further evaluation should be undertaken to determine the cause and need for specific treatment. *Fluid retention:* In a placebo-controlled trial of patients with severe chronic heart failure, there was an increased incidence of hospitalization for CHF associated with weight gain and increased leg edema during the first 4-8 weeks of treatment with TRACLEER®. In addition, there have been numerous post-marketing reports of fluid retention in patients with pulmonary hypertension, occurring within weeks after starting TRACLEER®. Patients required intervention with a diuretic, fluid management, or hospitalization for decompensating heart failure.

Information for Patients: Patients are advised to consult the TRACLEER® Medication Guide on the safe use of TRACLEER®. The physician should discuss with the patient the importance of monthly monitoring of serum aminotransferases and urine or serum pregnancy testing and of avoidance of pregnancy. The physician should discuss options for effective contraception and measures to prevent pregnancy with their female patients. Input from a gynecologist or similar expert on adequate contraception should be sought as needed.

Drug Interactions: CYP Isoenzymes: Bosentan is metabolized by CYP2C9 and CYP3A4. Inhibition of these isoenzymes may increase the plasma concentration of bosentan. Bosentan is an inducer of CYP3A4 and CYP2C9. Consequently, plasma concentrations of drugs metabolized by these isoenzymes will be decreased when TRACLEER® is co-administered. Contraceptives: Specific interaction studies have not been performed to evaluate the effect of co-administration of bosentan and hormonal contraceptives, including oral, injectable or implantable contraceptives. Since many of these drugs are metabolized by CYP3A4, there is a possibility of failure of contraception when TRACLEER® is co-administered. Women should not rely on hormonal contraception alone when taking TRACLEER®, Cyclosporine A. During the first day of concomitant administration, trough concentrations of bosentan were increased by about 30-fold. Steady-state bosentan plasma concentrations were 3- to 4-fold higher than in the absence of cyclosporine A. The concomitant administration of bosentan and cyclosporine A is contraindicated. Tacrolimus: Co-administration of tacrolimus and bosentan has not been studied in man. Co-administration of tacrolimus and bosentan resulted in markedly increased plasma concentrations of bosentan in animals. Caution should be exercised if tacrolimus and bosentan are used together. Glyburide: An increased risk of elevated liver aminotransferases was observed in patients receiving concomitant therapy with glyburide. Therefore, the concomitant administration of TRACLEER® and glyburide is contraindicated, and alternative hypoglycemic agents should be considered. Bosentan is also expected to reduce plasma concentrations of other oral hypoglycemic agents that are predominantly metabolized by CYP2C9 or CYP3A4. The possibility of worsened glucose control in patients using these agents should be considered. Ketoconazole: Co-administration of bosentan 125 mg b.i.d. and ketoconazole, a potent CYP3A4 inhibitor, increased the plasma concentrations of bosentan by approximately 2-fold. No dose adjustment of bosentan is necessary, but increased effects of bosentan should be considered. Simvastatin and Other Statins: Co-administration of bosentan decreased the plasma concentrations of simvastatin (a CYP3A4 substrate), and its active β-hydroxy acid metabolite, by approximately 50%. The plasma concentrations of bosentan were not affected. Bosentan is also expected to reduce plasma concentrations of other statins that have significant metabolism by CYP3A4, such as lovastatin and atorvastatin. The possibility of reduced statin efficacy should be considered. Patients using CYP3A4 metabolized statins should have cholesterol levels monitored after TRACLEER® is initiated to see whether the statin dose needs adjustment. Warfarin: Co-administration of bosentan 500 mg b.i.d. for 6 days decreased the plasma concentrations of both S-warfarin (a CYP2C9 substrate) and R-warfarin (a CYP3A4 substrate) by 29 and 38%, respectively. Clinical experience with concomitant administration of bosentan and warfarin in patients with pulmonary arterial hypertension did not show clinically relevant changes in INR or warfarin dose (baseline vs. end of the clinical studies), and the need to change the warfarin dose during the trials due to changes in INR or due to adverse events was similar among bosentan- and placebo-treated patients. Digoxin, Nimodipine and Losartan: Bosentan has been shown to have no pharmacokinetic interactions with digoxin and nimodipine, and losartan has no effect on plasma levels of bosentan.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Two years of dietary administration of bosentan to mice produced an increased incidence of hepatocellular adenomas and carcinomas in males at doses about 8 times the maximum

recommended human dose [MRHD] of 125 mg b.i.d., on a mg/m² basis. In the same study, doses greater than about 32 times the MRHD were associated with an increased incidence of colon adenomas in both males and females. In rats, dietary administration of bosentan for two years was associated with an increased incidence of brain astrocytomas in males at doses about 16 times the MRHD. Impairment of Fertility/Testicular Function: Many endothelin receptor antagonists have profound effects on the histology and function of the testes in animals. These drugs have been shown to induce atrophy of the seminiferous tubules of the testes and to reduce sperm counts and male fertility in rats when administered for longer than 10 weeks. Where studied, testicular tubular atrophy and decreases in male fertility observed with endothelin receptor antagonists appear irreversible. In fertility studies in which male and female rats were treated with bosentan at oral doses of up to 50 times the MRHD on a mg/m² basis, no effects on sperm count, sperm motility, mating performance or fertility were observed. An increased incidence of testicular tubular atrophy was observed in rats given bosentan orally at doses as low as about 4 times the MRHD for two years but not at doses as high as about 50 times the MRHD for 6 months. An increased incidence of tubular atrophy was not observed in mice treated for 2 years at doses up to about 75 times the MRHD or in dogs treated up to 12 months at doses up to about 50 times the MRHD. There are no data on the effects of bosentan or other endothelin receptor antagonists on testicular function in man.

Pregnancy, Teratogenic Effects: Category X

SPECIAL POPULATIONS: Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, breastfeeding while taking TRACLEER® is not recommended. Pediatric Use: Safety and efficacy in pediatric patients have not been established. Use in Elderly Patients: Clinical experience with TRACLEER® in subjects aged 65 or older has not included a sufficient number of such subjects to identify a difference in response between elderly and younger patients.

ADVERSE REACTIONS: Safety data on bosentan were obtained from 12 clinical studies (8 placebo-controlled and 4 open-label) in 777 patients with pulmonary arterial hypertension, and other diseases. Treatment discontinuations due to adverse events other than those related to pulmonary hypertension during the clinical trials in patients with pulmonary arterial hypertension were more frequent on bosentan (5%; 8/165 patients) than on placebo (3%; 2/80 patients). In this database the only cause of discontinuations > 1%, and occurring more often on bosentan was abnormal liver function. In placebo-controlled studies of bosentan in pulmonary arterial hypertension and for other diseases (primarily chronic heart failure), a total of 677 patients were treated with bosentan at daily doses ranging from 100 mg to 2000 mg and 288 patients were treated with placebo. The duration of treatment ranged from 4 weeks to 6 months. For the adverse drug reactions that occurred in ≥ 3% of bosentan-treated patients, the only ones that occurred more frequently on bosentan than on placebo (≥ 2% difference) were headache (16% vs. 13%), flushing (7% vs. 2%), abnormal hepatic function (6% vs. 2%), leg edema (5% vs. 1%), and anemia (3% vs. 1%).

Long-term Treatment: The long-term follow-up of the patients who were treated with TRACLEER® in the two pivotal studies and their open-label extensions (N=235) shows that 93% and 84% of patients were still alive at 1 and 2 years, respectively, after the start of treatment with TRACLEER®. These estimates may be influenced by the presence of eopostrenal treatment, which was administered to 43/235 patients. Without a control group, these data must be interpreted cautiously and cannot be interpreted as an improvement in survival.

Special Considerations: Patients with Congestive Heart Failure (CHF): Based on the results of a pair of studies with 1613 subjects, bosentan is not effective in the treatment of CHF with left ventricular dysfunction.

OVERDOSAGE: Bosentan has been given as a single dose of up to 2400 mg in normal volunteers, or up to 2000 mg/day for 2 months in patients, without any major clinical consequences. The most common side effect was headache of mild to moderate intensity. In the cyclosporine A interaction study, in which doses of 500 and 1000 mg b.i.d. of bosentan were given concomitantly with cyclosporine A, trough plasma concentrations of bosentan increased 30-fold, resulting in severe headache, nausea, and vomiting, but no serious adverse events. Mild decreases in blood pressure and increases in heart rate were observed. There is no specific experience of overdose with bosentan beyond the doses described above. Massive overdose may result in pronounced hypotension requiring active cardiovascular support.

DOSAGE AND ADMINISTRATION: TRACLEER® treatment should be initiated at a dose of 62.5 mg b.i.d. for 4 weeks and then increased to the maintenance dose of 125 mg b.i.d. Doses above 125 mg b.i.d. did not appear to confer additional benefit sufficient to offset the increased risk of liver injury. Tablets should be administered morning and evening with or without food.

Dosage Adjustment and Monitoring in Patients Developing Aminotransferase Abnormalities

ALT/AST levels	Treatment and monitoring recommendations
> 3 and ≤ 5 x ULN	Confirm by another aminotransferase test; if confirmed, reduce the daily dose or interrupt treatment, and monitor aminotransferase levels at least every 2 weeks. If the aminotransferase levels return to pre-treatment values, continue or re-introduce the treatment as appropriate (see below).
> 5 and ≤ 8 x ULN	Confirm by another aminotransferase test; if confirmed, stop treatment and monitor aminotransferase levels at least every 2 weeks. Once the aminotransferase levels return to pre-treatment values, consider re-introduction of the treatment (see below).
> 8 x ULN	Treatment should be stopped and reintroduction of TRACLEER® should not be considered. There is no experience with re-introduction of TRACLEER® in these circumstances.

If TRACLEER® is re-introduced it should be at the starting dose; aminotransferase levels should be checked within 3 days and thereafter according to the recommendations above. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin ≥ 2 x ULN, treatment should be stopped. There is no experience with the re-introduction of TRACLEER® in these circumstances. Use in Women of Child-bearing Potential: TRACLEER® treatment should only be initiated in women of child-bearing potential following a negative pregnancy test and only in those who practice adequate contraception that does not rely solely upon hormonal contraceptives, including oral, injectable or implantable contraceptives. Input from a gynecologist or similar expert on adequate contraception should be sought as needed. Urine or serum pregnancy tests should be obtained monthly in women of childbearing potential taking TRACLEER®. Dosage Adjustment in Renally Impaired Patients: The effect of renal impairment on the pharmacokinetics of bosentan is small and does not require dosing adjustment. Dosage Adjustment in Geriatric Patients: Clinical studies of TRACLEER® did not include sufficient numbers of subjects aged 65 and older to determine whether they respond differently from younger subjects. In general, caution should be exercised in dose selection for elderly patients given the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy in this age group. Dosage Adjustment in Hepatically Impaired Patients: The influence of liver impairment on the pharmacokinetics of TRACLEER® has not been evaluated. Because there is *in vivo* and *in vitro* evidence that the main route of excretion of TRACLEER® is biliary, liver impairment would be expected to increase exposure to bosentan. There are no specific data to guide dosing in hepatically impaired patients; caution should be exercised in patients with mildly impaired liver function. TRACLEER® should generally be avoided in patients with moderate or severe liver impairment. Dosage Adjustment in Children: Safety and efficacy in pediatric patients have not been established. Dosage Adjustment in Patients with Low Body Weight: In patients with a body weight below 40 kg but who are over 12 years of age the recommended initial and maintenance dose is 62.5 mg b.i.d. Discontinuation of Treatment: There is limited experience with abrupt discontinuation of TRACLEER®. No evidence for acute rebound has been observed. Nevertheless, to avoid the potential for clinical deterioration, gradual dose reduction (62.5 mg b.i.d. for 3 to 7 days) should be considered.

HOW SUPPLIED: 62.5 mg film-coated, round, biconvex, orange-white tablets, embossed with identification marking "62.5" NDC 66215-101-06; Bottle containing 60 tablets. 125 mg film-coated, oval, biconvex, orange-white tablets, embossed with identification marking "125" NDC 66215-102-06; Bottle containing 60 tablets.

Rx only.

STORAGE: Store at 20°C – 25°C (68°F – 77°F). Excursions are permitted between 15°C and 30°C (59°F and 86°F). [See USP Controlled Room Temperature].

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Manufactured by:
Pathon Inc.
Mississauga, Ontario, CANADA

Marketed by:
Actelion Pharmaceuticals US, Inc.
South San Francisco, CA



Table 2—Drugs Used to Treat Raynaud’s Phenomenon and Digital Ulcerations

Direct Vasodilators

- Calcium channel blockers
- Alpha adrenergic inhibitors
- Nitrate patches or nitroglycerin ointment
- ACE inhibitors
- Angiotensin receptor blockers
- Prostacyclin analogues
- PDE5 inhibitors

Perfusion-Enhancing/Thermoregulatory Agents

- Serotonin inhibitors
- Serotonin reuptake inhibitors
- Calcitonin gene-related peptide
- Pentoxifylline
- Cilostazol

Endothelial Integrity/Vascular Health

- Endothelin receptor blockers
- Fish oils
- Vitamin E, vitamin C
- Probucol, other statins
- Low-dose aspirin
- Anticoagulants

in critical ischemia and ulceration of the digits remains uncertain. A number of reports suggest that platelet dysfunction occurs in scleroderma and endothelial cell activation is prevalent. Certainly, the combination of vasospastic disease and intimal vascular damage and luminal narrowing lead to slowed blood flow and the opportunity for platelet adhesion and initiation of thrombotic events. There are reports that therapies that modulate platelet function are clinically useful and this may be a unifying mechanism for several effective agents.

Infection

Established ulceration is worsened and often made more painful as a result of infection of the underlying tissues. Chronic ulcers are almost always infected, at least superficially and sometimes into the deeper tissues. Infection generally responds well to antibiotics, although systemic rather than topical administration is preferred and higher dose plus more prolonged courses may be more effective as tissue levels of antibiotic are likely to be impaired by poor skin perfusion. Spread of infection into deeper tissue and to underlying bone leading to osteomyelitis is fortunately rare.

Trauma

A major factor in ulcer development in peripheral sites is local trauma. The initial injury can be trivial and it is an important aspect of patient education that should be addressed early in the disease. Skin moisturizers and barrier creams can be helpful to reduce the tendency to epithelial disruption in response to minor trauma. Avoidance of trauma and protective measures, such as the



Figure. (1) Digital ulcer (ischemic). (2) Digital ulcer (traumatic). (3) Histology of affected artery from amputation specimen.

use of gloves for gardening or the handling of abrasives, etc, are important even in warm surroundings. Traumatic ulcers may be especially troublesome over extensor surfaces of joints in association with contractures and atrophic skin. The fixed flexion contracture of the proximal interphalangeal joints in diffuse scleroderma is a common site of traumatic ulceration.

Calcinosis

Predominantly in patients with limited scleroderma, calcinotic deposits may underlie digital ulcers. These are readily identified by radiography and, when large, can be removed surgically, although local recurrence is the rule. Simple measures to improve skin texture and condition may prevent the development of skin breakdown over areas of calcinosis. At present there are no medical agents agreed to be effective in the reduction of calcinotic deposits, although low-dose warfarin, high-dose diltiazem, colchicines, and even minocycline have their advocates.

Management of Digital Ulceration and Critical Digital Ischemia

Treatment of established ulcers should address each of the mechanisms outlined above, with particular focus on the aspects that are relevant in individual cases. Raynaud’s treatment should be optimized. Response to oral vasodilators is variable and idiosyncratic, with different agents effective in different patients. Sometimes a combination approach is adopted using calcium

channel blockers, angiotensin converting enzyme inhibitors or angiotensin receptor blockade, direct vasodilators such as doxazosin, topical nitroglycerin ointment (not put on the hands but on the inner arms or trunk where the surface is more absorptive), and hemorheologic agents such as pentoxifylline or cilostazol. The maximum dose tolerated should be titrated according to blood pressure and side effects. Other agents that may be considered include selective serotonin reuptake inhibitors (SSRIs), which are often associated with fewer vasodilatory side-effects than directly vasoactive agents. While increasingly appreciated as effective, the mechanism of action of the SSRIs remains unclear but may involve reduction of conditioned reflex responses to circumstances likely to induce Raynaud attacks. Measures for general vascular health are inadequately studied but include statins, antioxidants, and fish oils.

A course of antibiotics should be tried for most chronic ulcers, preferably in early stages before there is substantial tissue damage. Agents such as dicloxacillin or flucloxacillin, cephalexins, or ciprofloxacin are first-line treatments in patients without a history of allergy or intolerance to these agents. Occasionally parenteral antibiotics may be required.

In cases of critical ischemia, where there is persistent digital discoloration and the affected digit does not return to normal color even with rewarming, more aggressive measures are indicated. Decisive and rapid clinical action is required. Parenteral prostacyclin derivatives have been used for many years for severe digital ulceration and critical ischemia. They are of proven efficacy in clinical trials and appear to benefit ulcer healing and may also prevent recurrence. In Europe parenteral iloprost (carbaprostacyclin) is available and widely used. In the United States epoprostenol (prostacyclin) has superseded prostaglandin E in treating critical ischemic events. Other parenteral prostacyclins, eg, treprostinil, have yet to be studied for this indication. Interestingly, the maximum thermographic benefit after a 5 sequential daily infusion of iloprost occurs as late as 6 weeks after treatment, suggesting a prolonged vascular effect in addition to any acute antiplatelet or vasodilator mechanism. Prostacyclin may be given for 3 to 5 days for ulceration and longer term low dose infusions may be used for critical digital ischemia or gangrene.

Other agents that have been reported useful include the phosphodiesterase 5 (PDE5) inhibitor, sildenafil. Doses from 25 mg to 50 mg orally q6-8h have been employed as a short-term treatment for critical digital ischemia although formal studies are lacking. Chronic sildenafil is difficult to obtain for patients and further clinical trials are necessary to confirm benefit. Other PDE5 inhibitors will soon be available as well.

Selective digital sympathectomy is a surgical technique that is

now well established as a treatment for a single ischemic digit with severe or painful ulceration. The operation involves dissection and stripping of the adventitia from the digital arteries at the base of a digit, which in itself may lead to improved arterial pulsatile flow. Good results have been reported, although controlled trials are lacking. Sometimes more proximal surgery, including ulnar artery bypass or ulnar and radial artery adventectomy, has been advocated. Appropriate candidates may be selected by pre-operative magnetic resonance angiography or by traditional contrast angiography.

Cervical sympathetic blocks, done on a daily basis or a constant epidural infusion of fentanyl/bupivacaine may also be helpful, as may cervical sympathectomy. The latter should be used only when there is serious threat of major tissue loss, such as digital infarction, as the effects are, in general, not long lasting and there are long-term side effects.

Future Treatments

Drawing analogy with the benefits of treatments for pulmonary arterial hypertension, there have been trials of the dual specificity endothelin receptor antagonist bosentan in scleroderma-associated digital ulceration. The results of the RAPIDS-1 trial were presented in abstract form at recent American College of Rheumatology meetings and suggest that new digital ulcers are prevented when patients are treated with bosentan. An improvement in net ulcer burden has also been demonstrated. There was no effect on healing of established ulcers and a further study, the RAPIDS-2 trial, is under way to examine this. For more information about this trial, see the Current Studies section of this journal or consult the SCTC Web page at <http://www.sctc-online.org>.

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RAPIDS-2

A **RA**ndomized, double-blind, **P**lacebo-controlled, multicenter study to assess the effect of bosentan on healing and prevention of **I**schemic **D**igital ulcers in patients with systemic **S**clerosis.

Study drug: Bosentan 125 mg b.i.d.

Design: Multicenter, randomized, double-blind, placebo-controlled, phase III study.

Sponsor: Actelion Pharmaceuticals Ltd.

Principal Investigators: J. Korn, Boston University, and J. Seibold, University of Medicine and Dentistry of New Jersey.

Patient Selection Criteria

Inclusion Criteria (main):

- Systemic sclerosis, diffuse or limited.
- Systemic sclerosis patients with at least one digital ulcer at baseline qualifying as a cardinal ulcer.
- Male or female patients >18 years of age.
 - Women of childbearing potential must have a negative pretreatment pregnancy test and use a reliable method of contraception during study treatment and for at least 3 months after study treatment termination.
 - Women not of childbearing potential are defined as postmenopausal (ie, amenorrhea for at least 1 year), or surgically or naturally sterile.

Exclusion Criteria (main):

- Digital ulcers due to conditions other than systemic sclerosis.
- Severe PAH (WHO class III and IV).
- Malabsorption or any severe organ failure (eg, lung, kidney, liver) or any life-threatening condition.
- Treatment with parenteral prostanoids (prostaglandin E, epoprostenol, or prostacyclin analogs) during the past 3 months prior to randomization.

- Treatment with inhaled, subcutaneous, or oral prostanoids one month prior to randomization.
- Previous treatment with bosentan.

Outcome Measures

Primary:

- Time to healing of the cardinal ulcer.
- Number of new digital ulcers during the treatment period.

Secondary:

- Hand functionality indices.
- Hand pain

Time Line

- Recruitment period: October 2003 – February 2004.
- Completion: 1st quarter 2005.
- Date of expected analysis: 2nd quarter 2005.

To date, one clinical trial, RAPIDS-1, has been performed in the same indication including scleroderma patients with or without digital ulcers at baseline. The RAPIDS-1 study showed that bosentan reduces significantly the number of new digital ulcers versus placebo. The safety profile of bosentan observed in the RAPIDS-1 study was similar to that observed in pulmonary hypertension, an indication currently approved in the countries where the RAPIDS-2 study is performed.

If you have a patient you think might be suitable, or if you would like more information about the study, please visit our Web site on www.sctc-online.org.

Centers involved (site participation depends on IRB/regulatory approval):

USA

University of Colorado Hospital, Denver, CO, Dr. Collier
Froedtert & Medical College, Milwaukee, WI, Dr. Csuka
Tulane University Health Sciences Center, New Orleans, LA, Dr. Doyle
University of Chicago Hospitals, Chicago, IL, Dr. Ellman
University of Alabama at Birmingham, Birmingham, AL, Dr. Fessler
UCLA School of Medicine, Los Angeles, CA, Dr. Furst
North Shore University Hospital, Manhasset, NY, Dr. Goldberg
University of Medicine and Dentistry of New Jersey, New Brunswick, NJ, Dr. Hsu
Thomas Jefferson University, Philadelphia, PA, Dr. Jimenez
Ruppert Health Center, Toledo, OH, Dr. Kahaleh
Spokane Rheumatology Group, Spokane, WA, Dr. Kenney
Boston University Medical Center, Boston, MA, Dr. Korn
Michigan State University, Grand Rapids, MI, Dr. Martin
University of Texas, Houston, TX, Dr. Mayes
University of Pittsburgh, Pittsburgh, PA, Dr. Medsger
Virginia Mason Research Center, Seattle, WA, Dr. Molitor
Mayo Clinic, Rochester, MN, Dr. Osborn
University of Connecticut Health Center, Farmington, CT, Dr. Rothfield
The Center for Rheumatology, Albany, NY, Dr. Shapiro
Medical University of South Carolina, Charleston, SC, Dr. Smith
Johns Hopkins University School of Medicine, Baltimore, MD, Dr. Wigley

Canada

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St. Joseph's Health Care London, London, Ontario, Dr. Pope
Hôpital Notre-Dame, Montreal, Quebec, Dr. Rich

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Hôpital Cochin, Paris, FR, Dr. Guillevin
Hôpital Pitie Salpetriere, Paris, FR, Dr. Frances
Centre Regional Hospitalier Universitaire, Lille, FR, Dr. Hachulla
Inselspital, Universitätsspital Bern, Bern, CH, Dr. Oertle
Universitätsklinik, Köln, GER, Dr. Krieg
Universitätsklinikum, Erlangen, GER, Dr. Manger
Universitätsklinikum, Dresden, GER, Dr. Meurer
Universitätsklinik, Freiburg, GER, Dr. Peter
Rheumaklinik, Bad Bramstedt, GER, Dr. Hellmich
Azienda Ospedaliera Carreggi, Firenze, IT, Dr. Matucci Cerinic
Ospedale Maggiore, Milano, IT, Dr. Scorza
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BUILD 2

Bosentan Use in Interstitial Lung Disease

A double-blind, randomized, placebo-controlled, multicenter study to assess the efficacy, safety, and tolerability of bosentan in patients with active interstitial lung disease associated with systemic sclerosis

Oral bosentan 125 mg / placebo b.i.d.

Primary endpoint: Change from baseline to month 12 in 6-minute walk distance.

Main secondary endpoint: Time to death or worsening pulmonary function tests.

Main Inclusion Criteria

- Systemic sclerosis diffuse or limited.
- Significant interstitial lung disease on HRCT scan.
- DLco <80% of predicted.
- Dyspnea on exertion.
- Walk not limited for musculoskeletal reasons.

Main Exclusion Criteria

- Interstitial lung disease due to conditions other than systemic sclerosis.
- End-stage restrictive or obstructive lung disease.
- Severe cardiac or renal diseases.
- Significant pulmonary arterial hypertension.
- Smoker (>5 cigarettes per day).
- Treatments with immunosuppressive, antifibrotic drugs, high dose corticosteroids (within 4 weeks of randomization).

Participating Sites

Site participation depends on IRB/regulatory approval

USA

- Medical Univ South Carolina, Charleston – Dr. Silver
- UMDNJ, New Brunswick – Dr. Seibold
- St Peters Hospital, Albany – Dr. Shapiro
- Virginia Mason Medical Center, Seattle – Dr. Molitor
- University of Washington, Seattle – Dr. Raghu
- Georgetown University, Washington, DC – Dr. Steen
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- Hutzel Hospital, Detroit – Dr. Chatterjee
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The Scleroderma Lung Study needs you and your patient!



This is a nationwide study to evaluate the effectiveness of oral cyclophosphamide versus oral placebo in the treatment of active, symptomatic lung disease (pulmonary alveolitis) due to scleroderma.

Subjects with fewer than 7 years of systemic sclerosis, who have some degree of shortness of breath and a forced vital capacity less than 85% of predicted, will have high-resolution chest computed tomography (HRCT) and bronchoalveolar lavage (BAL) to screen for active alveolitis. If positive for alveolitis, patients will be offered study medication for one year, with close follow-up for an additional year.

The goals of this study are to determine if lung function, quality of life, and the ability to function have improved with therapy. Subjects will receive study-related exams, lab tests, and medications at no cost.

If you think you have a potential patient, please call:

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