profiles of peripheral blood mononuclear cells to determine IFN signatures. Across the 16 different skin conditions there was an inverse correlation between IFN-β and IFN-γ, notably with particular diseases mapping to reproducible ratios—and thus offering molecular signposts to disease diagnostics. Collectively, the data provide valuable insight into the molecular processes that underlie various diseases and furthermore infer that similar processes underpin diseases from the same disease classifier.

Multi-disease classifiers, disease classification, and the development of personalized medicine

The core ethos underpinning the concept of personalized medicine is ''right patient, right drug, right dose, at the right time''. The integrative molecular classification, as well as the functional analysis, outlined in the study by Inkeles *et al.* (2014), offers a valuable tool in bringing personalized medicine closer to the clinical frontline. Although it is tempting to jump ahead and speculate about how multi-disease classifiers could be used to identify novel biomarkers and therapeutic targets for skin diseases, the current emphasis has to be on improving understanding of disease pathogenesis, implementing best clinical practice, and reflecting on whether current knowledge on disease classification and pathophysiology is matched by the multi-disease classifier data. Dermatology, and medicine in general, is littered with inappropriate (or frankly wrong) disease subgroupings. For example, 150 years ago epidermolysis bullosa and urticaria were thought to be similar diseases, and even in current practice, we still categorize different keratin genodermatoses as either mechanobullous diseases or forms of ichthyosis, despite the well characterized shared pathogenic overlap of keratin intermediate filament mutations and keratinocyte cytolysis. For inflammatory dermatoses, the dermatological literature contains numerous reports of imprecise entities such as ''psoriasiform eczema'' or ''eczematoid-lichenoid eruption'', but the data mining approach advocated by Inkeles *et al.* (2014) has the potential to help resolve such issues, or at least offer fresh insight into discrete or shared disease pathogeneses. However, perhaps stories of common disease pathogenesis only tend to become more relevant when therapies are introduced or challenged. The multi-disease classifier data presented by Inkeles *et al.* (2014) offer both a reality check and a glimpse into how personal diagnostics and therapeutics might develop. The study also highlights the considerable resources on gene expression data that are freely accessible in the public domain, and the value of thinking beyond the traditional disease-control experiment. There remains a rich seam of widely available skin disease gene signature data still available to mine, with significant potential benefits in store for the future classification and improved treatment of diseases in dermatology.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES

Inkeles M, Scumpia P, Swindell W *et al.* (2014) Comparison of molecular signatures from multiple skin diseases identifies mechanisms of immunopathogenesis. *J Invest Dermatol* 135:157–9

Kamsteeg M, Jansen PA, van Vlijmen-Willems IM *et al.* (2010) Molecular diagnostics of psoriasis, atopic dermatitis, allergic contact dermatitis and irritant contact dermatitis. *Br J Dermatol* 162:568–78

Kawasaki ES (2005) The end of the microarray Tower of Babel: will universal standards lead the way? *J Biomol Tech* 17:200–6

Lee JH, Daugharthy ER, Scheiman J *et al.* (2014) Highly multiplexed subcellular RNA sequencing *in situ*. *Science* 343:1360–3

Lockhard DJ, Dong H, Byrne MC *et al.* (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* 14:1675–80

Schena M, Shalon D, Davis RW *et al.* (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:368–71

Shalek AK, Satija R, Shuga J *et al.* (2014) Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. *Nature* 510:363–9

Wang Z, Gerstein M, Snyder M (2009) RNA-seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10:57–63

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Healing Refractory Venous Ulcers: New Treatments Offer Hope

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Non-healing wounds are associated with an inflammatory and proteolytic wound environment, and recent therapeutic strategies have been focused on reversing these changes. Connexins, as members of gap junctions, are important in intercellular signaling and wound repair. Connexin 43 (Cx43) downregulation is associated with normal wound healing, and it has been found to be upregulated in non-healing venous leg ulcers (VLUs). Ghatnekar *et al.* (2014) report findings of a small phase II trial performed in Indian patients with chronic VLUs, reporting that ACT1, a mimetic peptide of Cx43, accelerates healing in the treatment group. Despite standard care with compression therapy and adjuvant therapy for refractory wounds, at present in clinical practice a significant number of patients remain unhealed. The potential for ACT1 exists to help heal refractory VLUs, but it faces additional regulatory hurdles.

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Clinical Implications

- Despite appropriate standard of care including multilayered compression wrappings, more than a quarter of patients with VLUs fail to heal.
- Connexin 43 (Cx43), a component of gap junctions, has an important role in many aspects of wound healing, and Cx43 is upregulated in wound edges of nonhealing VLUs.
- ACT1, a mimetic peptide of Cx43, in a Phase 2 study accelerated healing of refractory VLUs compared with a regimen of standard of care.

Over a quarter of patients with venous leg ulcers (VLUs) fail to heal with standard care, and even those who do heal often take 6 months or longer to do so (de Araujo *et al.*, 2003). New treatments for VLUs are needed. In this issue of the *Journal* Ghatnekar *et al.* (2014) describe a novel treatment that employed ACT1 (First String Research, Mt Pleasant, SC)—a connexin 43 (Cx43) peptide mimetic of the carboxyl-terminus of Cx43. Although this study shows promise (Ghatnekar *et al.*, 2014), it is important to put these results into context by reviewing where we are and where we are going in treating VLUs.

The importance of VLUs

VLUs are the most common cause of leg ulcers, affecting up to 1% of all adults and 3% of older adults because of its increasing incidence and prevalence with advancing age. The average annual incidence rate of VLU is 2.2% in Medicare aged populations and 0.5% in younger patients (Rice *et al.*, 2014). VLUs are associated with impaired venous return, caused by calf muscle pump dysfunction. This is most often due to obstruction or valvular dysfunction affecting superficial, perforator, or deep veins, and it leads to sustained ambulatory venous pressures (also known as venous hypertension; Figure 1). As a result, patients develop edema and slow healing wounds, most commonly, on their lower legs or ankles (Yim *et al.*, 2014).

Accounting for upward of 90% of leg ulcers, VLUs are associated with reduced quality of life (QOL) and increased healthcare costs. For example, recent data for both young (privately insured) and older (Medicare insured) patients with VLUs indicate that patients with VLUs utilize significantly more medical resources and have increased annual incremental medical costs compared with matched patients without VLUs (Rice *et al.*, 2014). Working patients with VLUs missed more days from work, resulting in substantially higher work-loss costs.

Not only is QOL reduced in patients with VLUs, treatment improves health-related QOL (Salome *et al.*, 2014). After excluding concomitant significant arterial disease, standard care most often employs multilayered compression wraps, which are intended to reverse the pathogenic mechanism. Effective compression therapy results in healing in up to three quarters of patients with VLUs, especially in those with small ulcers that are of short duration.

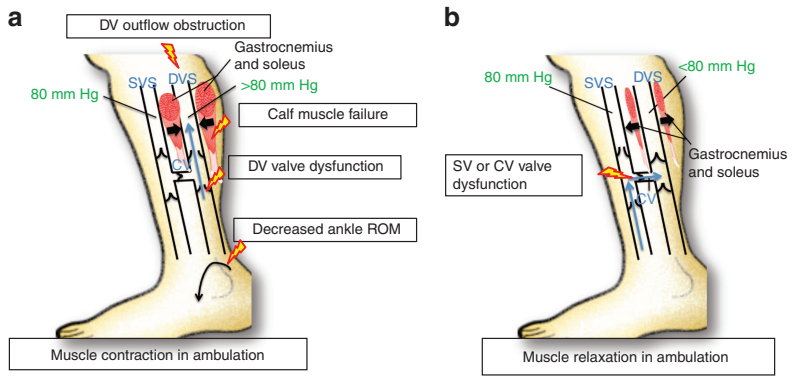

Figure 1. Calf muscle pump and venous insufficiency. Venous blood return to the heart results from venous blood flowing from the SVS to the DVS via the action of the calf muscle pump. At rest in the standing position, the hydrostatic pressure in the SVS and DVS systems is approximately 80 mm Hg and net flow equals 0. (**a**) Muscle contraction during ambulation. With full range of movement (ROM) of the ankle during ambulation and resultant contraction of the gastrocnemius and soleus muscles, a pressure >80 mm Hg is exerted on the DVS and venous blood flows cephalad, whereas the SVS and CV valves close to prevent retrograde flow into the SVS. (**b**) Muscle relaxation in ambulation. With relaxation of calf muscles following the emptying of the DVS, pressure therein decreases below 80 mm Hg and blood from the SVS empties into the DVS through the patent SV and CV valves. A combination of pathologies may occur resulting in venous insufficiency. SVS or CV valve dysfunction, calf muscle failure, decreased ROM at the ankle, DVS outflow obstruction, and DVS valve dysfunction can all cause venous hypertension or more appropriately termed sustained ambulatory venous pressures (venous pressure that does not reduce with walking). CV, communicating veins; DVS, deep venous system; SV, superficial veins; SVS, superficial venous system. Arrows toward DVS, calf muscle contraction; arrows away from DVS, calf muscle relaxation. Blue arrows, venous flow.

Approach to standard and adjuvant care of VLUs

Clinical guidelines encourage a 4-week trial of standard care, and, if significant reduction in wound size does not occur (40% in typical ulcers $<10\,cm^2$) during this time, adding adjuvant care to compression therapy is recommended (Tang *et al.*, 2012). Data from randomized control trials for commercially available products suggest adding oral agents, such as Pentoxifylline (up to 800 mg three times daily) or Aspirin, or topically applied cell and tissue-based products, such as cellular products, including a bilayer living skin equivalent (Apligraf, Organogenesis, Canton, MA) or acellular constructs such as porcine small intestine submucosa (Oasis, Smith and Nephrew, Largo, FL) or poly-N-acetyl glucosamine isolated from microalgae (pGIcNAc, Talymed, Marine Polymer Technologies, Danvers, MA). A recent comparative effectiveness study found better healing with a bilayer living skin equivalent compared with the porcine small intestine submucosa in clinical practice

(Marston *et al.*, 2014). Topical growth factors to speed up healing of VLUs are not available commercially, but intralesional injection of GM-CSF, available as a hematopoiesis stimulant, was effective in healing VLUs as an adjuvant to compression in a phase II trial. Nonetheless, its use is limited by local and systemic (bone) pain associated with injections (Richmond *et al.*, 2013; Table 1).

Despite a long history of venous surgical interventions to speed up VLU healing, data to date do not demonstrate improved healing of existing ulcers, but they do suggest a decrease in both severity and rates of recurrence after surgical intervention (Gohel *et al.*, 2007). More invasive surgery with high complication rates have been replaced by less invasive percutaneous procedures such as radiofrequency, endovascular laser ablation, and ultrasound guided foam sclerotherapy. Enthusiasm exists for these procedures, and time will tell whether clinical data match that enthusiasm (Siribumrungwong *et al.*, 2012).

New strategies for refractory VLUs

A number of technologies are currently under investigation for treating VLUs; cell-based therapies are farthest along, in phase III studies. HP-802 (Smith and Nephew, Fort Worth, TX) is a novel therapy of spray applied cell therapy containing growth arrested allogeneic neonatal keratinocytes and fibroblasts. phase II dose finding studies have found an optimal dose and a statistical and clinical benefit with increased and faster healing when given every 14 days via a spray technology (Kirsner *et al.*, 2012). Another technology, allogeneic white blood cells delivered by periodic intralesional injection (CureXcell, Macrocure, Petach Tikva, Israel), which has been used extensively in Israel, based on non-randomized control data, is now being studied in the United States in a phase III trial (Zuloff-Shani *et al.*, 2010).

Given this background information, we address new data for ACT1. Ninety-two Indian patients with refractory VLUs were randomized to one of the two parallel groups—either weekly ACT1 gel formulation application plus four-layer compression bandage therapy or four-layered compression. The use of ACT1 was associated with a significantly greater reduction in mean percent ulcer area during the 12-week study (79 vs. 36%, $P=0.02$) and greater wound closure at week 12 (57 vs. 28%, $P=0.006$). No safety concerns were noted, albeit it was a small phase II trial.

Rationale for new strategies for VLU treatment

Why venous ulcers fail to heal despite adequate compression is not known. What is known is that with prolonged non-healing of wounds, cellular and molecular changes occur within the wound bed and at wound edges (Charles *et al.*, 2008). An inflammatory and proteolytic wound environment, coupled with molecular changes at the wound edges likely contribute to poor healing. Reversing these changes represents a ''therapeutic opportunity.''

Connexins are the channel-forming component of gap junctions (GJs) that allow small molecule exchange (<1000 Da) and facilitate signaling and electrical impulse propagation. Given the importance of GJs, it would be expected that Connexins, including Cx43, would have many functions, in all phases of wound healing (and especially during the inflammatory and proliferative phases). Cx43 has critical roles in regulating the inflammation, edema, and fibrosis that follow tissue injury. During normal wound healing, Cx43 downregulation is correlated with increases in transforming growth factor-β mRNA and collagen α-1 and with decreases in chemokine ligand-2 and tumor necrosis factor-α expression. Cx43 downregulation thereby promotes angiogenesis, fibroblast migration, keratinocyte proliferation, and migration, and it decreases the number of infiltrating neutrophils and macrophages at sites of wounding (Mori *et al.*, 2006). In chronic VLUs, Cx43 is upregulated abnormally at the epidermal wound margins (Mendoza-Naranjo *et al.*, 2012). ACT1 is a 25aa peptide containing the carboxy-terminal PDZ-binding domain that selectively inhibits Cx43, independent of Cx43 expression, affecting Cx43 hemichannels (Ghatnekar *et al.*, 2009). Hemichannels provide a paracrine route for intercellular signaling and communication and, as a result, regulate wound-healing processes associated with inflammation, edema, and fibrosis. Reduction in Cx43 hemichannel activity tempers inflammatory responses, shortens the inflammatory phase, and potentially improves healing. Targeting Cx43 with polynucleotide antisense DNA has led to accelerated fibroblast migration and proliferation (Mendoza-Naranjo *et al.*, 2012).

Clinical potential of ACT1 and pitfalls of phase III trials

Although the data for ACT1 are exciting, development of a new treatment, especially using drugs, is a rigorous process. Only 1 out of every 5,000 pharmaceutical compounds evaluated reach United States Food and Drug Administration approval, and only about one in four drugs that is successful in phase II trials gains approval after successful completion of phase III trials. High costs and large amounts of time are invested, with development costs for a drug containing a novel new chemical entity estimated to be between $600 and $800 million dollars (Maderal *et al.*, 2012). While this cost reflects not only direct costs of the development of the drug and clinical trials, it also includes the development costs of failures; costs for a typical phase III trial for VLU is expensive, between $5 and $15 million. As a result, start-up companies may find funding of well-done studies to be problematic. Failure to appreciate trial costs can lead to under-budgeting and fiscal stress, as trials last longer and cost more than is commonly predicted. Often, under-budgeting is a consequence of taking the most optimistic opinions regarding patient enrollment. When this occurs, poorly selected clinical sites may be recruited, and, after the study begins, inclusion and exclusion criteria tend to be modified to speed up enrollment, often leading to less homogenous and perhaps inappropriate populations and perhaps a less rigorous standard care being applied. This can affect the quality of the study, and it favors finding no difference in outcomes between treatment groups. Therefore, instead of studying a discrete subset of patients most likely to benefit, industry sponsors may broaden the inclusion criteria on the basis of

Table 1. Adjuvant therapies for venous leg ulcers

Administration	Agent	Mechanism of action	Supporting data	Comments
Systemic	Pentoxifylline	Lowers leukocyte adhesion to vascular endothelium Increases collagenase expression Tumor necrosis factor-α effect antagonist	A recent Cochrane review found that pentoxifylline was superior to placebo in achieving ulcer healing in VLU (RR, 1.70; 95% CI, 1.30–2.24), both used as adjuvant to compression (RR, 1.56; 95% CI, 1.14–2.13) and without compression (RR, 2.25; 95% CI, 1.49–3.39; Jull *et al.*, 2012).	Higher doses (800 mg per day) provide greater benefit
	Aspirin	Reduces inflammation Inhibits platelet coagulation	A prospective randomized parallel controlled intention-to-treat trial (51 VLU patients) found that 300 mg oral aspirin, as adjuvant to standard compression therapy, decreased time to heal by 46% (12 weeks vs. 22 weeks in the control group; the log-rank test $\chi^2_1 = 3.90$, $P = 0.04$; del Rio Sola *et al.*, 2012).	
Tissue engineered cellular products	Apligraf. Human growth–arrested keratinocytes and fibroblasts bovine type I collagen	Increases concentration of growth factors and cytokines	Especially effective in refractory venous ulcers. Subset analysis of a RCT (120 VLU patients) found that bilayered living skin construct (Apligraf) plus compression therapy increased healing rates in wounds greater than 1 year duration compared with compression therapy alone (6-month healing rates 47% vs. 19% of patients, $P < 0.005$). By 6 months patients in the intervention group were more than two times as likely to completely heal (Falanga and Sabolinski, 1999).	Comparative effectiveness data suggested that Apligraf promotes superior healing compared with porcine small intestine submucosa
Acellular products	Oasis. Porcine small intestine submucosa	Promotes angiogenesis Increases tendency for host cell infiltration and growth	A multicenter RCT (120 patients with VLU) found that after 12 weeks of therapy 55% of wounds treated with Oasis healed as opposed to 34% in the group with only compression therapy ($P = 0.0196$; Mostow *et al.*, 2005).	
	Poly-N-acetyl glucosamine (pGlcNAc; Talymed)	Promotes angiogenesis and granulation tissue development Triggers rapid epithelialization	A randomized, investigator-blinded, parallel-group, controlled study (four groups, total $N = 82$ patients) found that pGlcNAc applied every other week healed 86.4 versus 45% of patients treated with standard care alone (Kelechi *et al.*, 2012).	
Growth factors	GM-CSF	Triggers keratinocyte proliferation Activates endothelial cell and fibroblast differentiation	A double-blind RCT (60 VLU patients) found that perilesional injections of GM-CSF 200 μg and 400 μg at 13 weeks healed 57 and 61% patients, respectively, compared with 19% of patients in the placebo group ($P = 0.05$; Da Costa *et al.*, 1999).	Available as hematopoiesis stimulant
Surgical intervention	Venous surgery	Ameliorates venous insufficiency Decreases venous reflux	A RCT (500 VLU patients) did not show improved healing in VLUs treated with saphenous surgery in addition to compression therapy ($P = 0.73$, log-rank test). However, patients who underwent surgery experienced a significant decrease in ulcer recurrence rates at 4 years compared with patients who only had compression therapy (31% vs. 56%, $P < 0.01$; Gohel *et al.*, 2007).	
Minimally invasive procedures	Radio frequency Endovascular laser ablation Ultrasound guided foam sclerotherapy	Improves venous circulation through varicose vein ablation		No randomized controlled trials to date

Abbreviations: CI, confidence interval; GM-CSF, granulocyte-macrophage colony stimulating factor; RCT, randomized controlled trial; RR, relative risk; VLU, venous leg ulcer.

pragmatic or even market considerations as opposed to scientific rationale.

Summary

Novel treatments for VLUs are needed. Algorithms of care exist for the management of patients with VLUs, but still a significant subset of patients have ulcers that remain unhealed. As non-healing wounds are associated with an inflammatory and proteolytic wound environment, recent strategies have been focused on reversing these changes. Cx43 downregulation is associated with normal wound healing, and it has been found to be upregulated in non-healing VLUs. Results of the use of ACT1, a mimetic peptide of Cx43, in the treatment for refractory VLUs hold promise.

CONFLICT OF INTEREST

Drs Kirsner and Baquerizo Nole and Mr Fox are investigators through the University of Miami for Smith and Nephew (HP802) and Macrocure (CureXcell). Drs Kirsner and Baquerizo Nole were investigators through the University of Miami for CoDA Therapeutics, an unrelated company testing an inhibitor of connexin 43 expression.

REFERENCES

Charles CA, Tomic-Canic M, Vincek V *et al.* (2008) A gene signature of nonhealing venous ulcers: potential diagnostic markers. *J Am Acad Dermatol* 59:758–71

Da Costa RM, Ribeiro Jesus FM, Aniceto C *et al.* (1999) Randomized, double-blind, placebo-controlled, dose- ranging study of granulocyte-macrophage colony stimulating factor in patients with chronic venous leg ulcers. *Wound Repair Regen* 7:17–25

de Araujo T, Valencia I, Federman DG *et al.* (2003) Managing the patient with venous ulcers. *Ann Intern Med* 138:326–34

del Río Solá MI, Antonio J, Fajardo G *et al.* (2012) Influence of aspirin therapy in the ulcer associated with chronic venous insufficiency. *Ann Vasc Surg* 26:620–9

Falanga V, Sabolinski M (1999) A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen* 7:201–7

Ghatnekar G, Grek C, Armstrong DG *et al.* (2014) The effect of a connexin43-based peptide on the healing of chronic venous leg ulcers: a multicenter, randomized trial. *J Invest Dermatol* 135:289–98

Ghatnekar GS, O'Quinn MP, Jourdan LJ *et al.* (2009) Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. *Regen Med* 4:205–23

Gohel MS, Barwell JR, Taylor M *et al.* (2007) Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): randomised controlled trial. *Br Med J* 335:83

Jull AB, Arroll B, Parag V *et al.* (2012) Pentoxifylline for treating venous leg ulcers. *Cochrane Database Syst Rev* 12:CD001733

Kelechi TJ, Mueller M, Hankin CS *et al.* (2012) A randomized, investigator-blinded, controlled pilot study to evaluate the safety and efficacy of a poly-N-acetyl glucosamine-derived membrane material in patients with venous leg ulcers. *J Am Acad Dermatol* 66: e209–15

Kirsner RS, Marston WA, Snyder RJ *et al.* (2012) Spray-applied cell therapy with human allogeneic fibroblasts and keratinocytes for the treatment of chronic venous leg ulcers: a phase 2, multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* 380: 977–85

Maderal AD, Vivas AC, Eaglstein WH *et al.* (2012) The FDA and designing clinical trials for chronic cutaneous ulcers. *Semin Cell Dev Biol* 23:993–9

Marston WA, Sabolinski ML, Parsons NB *et al.* (2014) Comparative effectiveness of a bilayered living cellular construct and a porcine collagen wound dressing in the treatment of venous leg ulcers. *Wound Repair Regen* 22:334–40

Mendoza-Naranjo A, Cormie P, Serrano AE *et al.* (2012) Targeting Cx43 and N-cadherin, which are abnormally upregulated in venous leg ulcers, influences migration, adhesion and activation of Rho GTPases. *PLoS One* 7:e37374

Mori R, Power KT, Wang CM *et al.* (2006) Acute downregulation of connexin43 at wound sites leads to a reduced inflammatory response, enhanced keratinocyte proliferation and wound fibroblast migration. *J Cell Sci* 119:5193–203

Mostow EN, Haraway GD, Dalsing M *et al.* (2005) Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial. *J Vasc Surg* 41:837–43

Rice JB, Desai U, Cummings AK *et al.* (2014) Burden of venous leg ulcers in the United States. *J Med Econ* 17:347–56

Richmond NA, Maderal AD, Vivas AC (2013) Evidence-based management of common chronic lower extremity ulcers. *Dermatol Ther* 26:187–96

Salome GM, Blanes L, Ferreira LM (2014) The impact of skin grafting on the quality of life and self-esteem of patients with venous leg ulcers. *World J Surg* 38:233–40

Siribumrungwong B, Noorit P, Wilasrusmee C *et al.* (2012) A systematic review and meta-analysis of randomised controlled trials comparing endovenous ablation and surgical intervention in patients with varicose vein. *Eur J Vasc Endovasc Surg* 44:214–23

Tang JC, Marston WA, Kirsner RS (2012) Wound Healing Society (WHS) venous ulcer treatment guidelines: what's new in five years? *Wound Repair Regen* 20:619–37

Yim E, Richmond NA, Baquerizo K *et al.* (2014) The effect of ankle range of motion on venous ulcer healing rates. *Wound Repair Regen* 22:492–6

Zuloff-Shani A, Adunsky A, Even-Zahav A *et al.* (2010) Hard to heal pressure ulcers (stage III-IV): efficacy of injected activated macrophage suspension (AMS) as compared with standard of care (SOC) treatment controlled trial. *Arch Gerontol Geriatr* 51:268–72