


Clinical Protocol: Phase I trial to evaluate the safety of H5.020CMV.PDGF-B for the treatment of a diabetic insensate foot ulcer

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Most patients with chronic wounds fail to heal in a reasonable period of time. Despite considerable advances in elucidating the molecular basis of wound repair, attempts at developing new therapies have been disappointing. In fact, in the few studies where cytokine growth factors have been efficacious, their effect has been dramatically less than would have been predicted from animal studies. We hypothesize that platelet-derived growth factor-BB, a growth factor associated with wound healing, when produced in large quantities within the wound bed due to adenovirus mediated gene overexpression by the cells of the wound bed will dramatically enhance wound healing. Simply stated, we plan to insure the delivery of the growth factor by using gene therapy techniques so that cells locally involved in the wound healing process will temporarily increase their production of platelet-derived growth factor-BB. We present the first step in the series of human investigations to test this hypothesis which is a phase I clinical trial. Our proposed study is designed to assess local and systemic toxicity, and the feasibility of using the maximum tolerated dose of H5.020CMV.PDGF-b associated with in vivo platelet-derived growth factor-BB gene transduction via an intraulcer injection of H5.020CMV.PDGF-b in patients with a diabetic insensate foot ulcer. **(WOUND REP REG 2000;8:480-493)**

Lower extremity ulcers are a serious complication of diabetes mellitus. In the past few years there have been considerable advances in elucidating the molecular basis of wound repair and many potential agents have been examined for vulnerary activity. Many of these newly described agents are cytokines and their effect on wound healing in preclinical animal studies has been impressive. However, attempts at developing new human therapies have been disappointing. To date only one cytokine growth factor, platelet-derived growth factor (PDGF-

BB), has been approved for use on diabetic insensate foot ulcers and its effect on healing wounds is moderate. No other cytokine has been shown to be efficacious. Why the effect of cytokine growth factors in healing human chronic wounds has been disappointing is not well described. It is possible that in the human trials cytokine growth factors have not been efficiently delivered to the cells needed to heal the wound.

We propose to improve the efficacy of a cytokine growth factor for the treatment of human chronic wounds by gene transfer of PDGF-BB into the cells in-

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AE	Adverse event
DLT	Dose limiting toxicity
FDA	Food and Drug Administration
MTD	Maximum tolerated dose
PDGF	Platelet-derived growth factor
PFU	Plaque forming unit
RAC	Recombinant DNA Advisory Committee
rh PDGF	Recombinant human PDGF
SAE	Serious AE

volved in wound healing. To this end, we will use an adenovirus vector that is capable of efficiently transducing post-mitotic cells. We selected PDGF-BB because it is a potent chemoattractant and mitogen for many of the cells involved in wound repair and has already been shown to be safe and moderately effective for the treatment of diabetic insensate foot ulcers. In addition, pre-clinical studies have shown that the effect of PDGF-BB is dramatically enhanced when it is produced in the wound bed after it is applied using an adenoviral vector (PDGF-B/Ad5) as compared to its wound healing effects when PDGF-BB is applied topically. We believe that this unique and innovative approach, using gene therapy induced production of PDGF-BB and good wound care, will dramatically augment wound healing. The following protocol is the first step in testing our hypothesis. It is a phase I study designed to determine the safety and biologic feasibility of using this therapy.

This proposed trial was presented in December of 1999 to the Office of Biotechnology Activities of the National Institutes of Health (aka RAC [Recombinant DNA Advisory Committee]). It was the first such study presented to and registered with the RAC that uses a gene therapy approach to investigate a human chronic wound. This study was well received by the RAC reviewers (Minutes of Symposium and Meeting, Recombinant DNA Advisory Committee December 8-10, 1999 <http://www4.od.nih.gov/oba/1299rac.pdf>). The study and consent form has received appropriate approvals from the local institutional review boards at the University of Pennsylvania, Wistar Institute, and Children's Hospital of Philadelphia. However, final Food and Drug Administration (FDA) Investigational New Drug approval has not yet been received. The FDA Investigational New Drug application has been prepared and will be formally submitted as soon as all the well-publicized FDA "clinical hold" issues concerning gene therapy research at the University of Pennsylvania have been rectified. This is expected to occur before the end of summer 2001.

CLINICAL PROTOCOL SUMMARY

Despite considerable advances in elucidating the molecular basis of wound repair, attempts at developing new therapies have been disappointing. In general, as compared to standard care, new therapies based on recently elucidated mechanisms of wound repair have shown modest improvements in the overall number of individuals with a treated healed chronic wound. In fact, in the few studies where cytokine growth factors have been efficacious, the efficacy as compared to standard care

has been dramatically less than would have been predicted from animal studies. The proposed phase I clinical trial will focus on the use of the cytokine growth factor PDGF-BB. This growth factor works dramatically in animal studies and has recently been shown to have a modest effect in humans with diabetic foot ulcers, a type of chronic wound. PDGF-BB will be produced in the wound by cells in the wound as a result of PDGF-BB gene over-expression induced by adenoviral gene transfection. Simply stated, we plan to insure the delivery of growth factor by using gene therapy techniques to insure that cells involved in the wound healing process locally increase the production of PDGF-BB. We will investigate the acute safety of this technique and its effect on diabetic foot ulcers.

INTRODUCTION

Lower extremity ulcers are a serious complication of diabetes mellitus. More than 16 million people in the US have diabetes mellitus and 15% of them can expect to develop a foot ulcer at some point in their life.¹⁻⁷ The annual incidence of foot ulcers in community-based studies on diabetics is between 2 and 3% with a prevalence of 4-10%.^{2,4} For hospitalized patients, data from the 1983-90 National Hospital Discharge survey indicates that 6% of all diabetics were coded on hospital discharge as having a lower extremity ulcer.² While only 4% of the population has diabetes mellitus, 46% of those admitted to a hospital with a foot ulcer had diabetes and half of all lower extremity amputations in hospitalized patients occurred in diabetics.^{4,8-10} Diabetics admitted to the hospital with lower extremity ulcers were, on average, hospitalized longer than diabetics who were hospitalized and did not have ulcers.⁸⁻¹⁰ Finally, it has been well established that those with a lower extremity amputation have a diminished quality of life, increased health costs, often have many concomitant medical ailments, are more likely to have the contralateral limb amputated than those without an amputation, and are more likely to die within the next five years than those who have not had an amputation.^{8,9,11-17}

This is an exciting time for clinicians interested in treating patients with chronic wounds. In the past few years, newer surgical techniques and several novel biotechnology-based treatment modalities have been proposed to treat patients with diabetic foot ulcers.¹⁸⁻²¹ In fact, this is especially true for patients with diabetic neuropathic foot ulcers. For this complication of diabetes, in just the past two years the FDA has approved two very different therapies. One therapy is a skin substitute

that is expected to be available in the first quarter of 2001.¹⁸ The other is recombinant human PDGF (rhPDGF), which was the first growth factor approved for the treatment of a human chronic wound;^{19,20} Both of these treatments were evaluated specifically in diabetics with neuropathy and sufficient lower limb arterial blood flow.^{18–20}

Diabetic foot ulcer

There are many etiologic pathways for the development of a diabetic foot ulcer.²² The majority of these pathways include some combination of lower limb arterial insufficiency, lower limb diabetic neuropathy, and local trauma. In fact, for the past several years it has been hypothesized that individuals with diabetes develop foot ulcers due to two basic problems, an inability to protect their feet from the physical trauma of daily living due to neuropathy, or insufficient arterial blood flow to the foot.^{5,10,23–27} In general, about 20% of diabetics with foot ulcers primarily have inadequate arterial blood flow, about 50% primarily have diabetic neuropathy, and about 30% are afflicted with both conditions.^{2,10,22} Inadequate arterial blood flow is primarily treated by a variety of surgical techniques that improve blood flow. Surgical techniques have improved remarkably in the past 10 years.^{28–32} Individuals who lack protective sensation and have adequate arterial blood flow to their foot will be called diabetics with a neuropathic foot ulcer in this proposal.^{18,23}

Therefore, the treatment of a diabetic with a foot ulcer begins with a work-up that should be focused on determining if there is adequate lower extremity arterial perfusion.^{1,18,19,21,33–37} In general, a limb is considered to have arterial blood flow adequate for healing, if $T_rPO_2 > 30\text{--}40$ mm of Hg, which is a measure of local blood oxygenation, or an ankle brachial index is greater than 0.80, which is a measure of blood pressure in the lower limb, or if a palpable dorsal pedal or posterior tibial pulse can be appreciated.^{6,23,28,38,39} All patients should also be evaluated for lower limb sensation. This is often done using Semmes-Weinstein 5.07 (10-gram) monofilament or a Biothesiometer applied to several different sites on the foot.^{37,40,41} The Semmes-Weinstein 5.07 filament is more frequently used in the clinical setting and correlates well with Biothesiometer measurements.^{40–42} It has been shown in the past that an inability to perceive 10 g of pressure correlates with an inability to perceive enough sensation to protect the foot from ulceration.^{25,37} To treat the neuropathic wound most experts extensively débride the wound, use a moist dressing, and then provide a device so that the wound no longer bears pressure or is physically traumatized. It is the very act of walking and bearing weight on the foot that causes the ulcer and may

keep it from healing. Off loading is a term used to describe many different techniques used to prevent the foot and wound from being repeatedly traumatized. Several devices exist and are commonly used including contact cast, crutches, wheel chairs, and special footwear.^{1,19,20,33,28,37,43} This type of standard care has been used as the standard care arm in several recent randomized clinical trials and was recently discussed in a consensus statement from the American Diabetes Association,^{18–20,28,43}

With the advent of biotechnology several new treatments have been introduced. These products include topical rhPDGF, skin substitutes, topical collagen, electric stimulation, cold laser, topical platelet releasate, and hyperbaric oxygen,^{18–21,44–56} However, only a few treatments have received FDA approval for the treatment of a lower extremity chronic wound, namely a cultured skin substitute and rh PDGF.^{18–20} However, even with the advent of these new products, success in treating diabetic neuropathic foot ulcers is dismal. Approximately 33% of the patients in the standard care arms of clinical trials will heal by 20 weeks of care while approximately 43% of the individuals that receive one of the new products will heal by 20 weeks of care.^{18–20}

PDGF— Wound effects

The use of PDGF as an agent to augment wound healing is based on several lines of evidence. Early in the inflammatory phase of wound healing during the incorporation of a platelet into the fibrin plug, platelets release the contents of their alpha granules. Since PDGF is present in the alpha granules of platelets, it is present early in the wound reparative process and in large quantities.^{51,52} PDGF is also secreted by several other types of cells active in wound repair such as: macrophages, endothelial cells, fibroblasts, and keratinocytes.^{53,54} In fact, in the presence of PDGF macrophages are stimulated to produce and secrete transforming growth factor- β , vascular endothelial cell growth factor, and more PDGF. Transforming growth factor- β and vascular endothelial cell growth factor are also believed to be important in wound healing. In vivo PDGF has been shown to be an important mitogen for fibroblasts and stimulates fibroblasts to produce extracellular matrix.^{51,52,55} Finally, PDGF and PDGF receptors are found in wounds and in cells actively involved in wound repair.^{56,57}

Several studies have also indicated that PDGF, when applied to the wounds of animals, increases the rate with which the wounds heal and increases the strength of the healed wound (scar). The studies have generally applied PDGF topically in a liquid or gel formulation. The application of PDGF to the surface of a wound does not mimic its release from platelets, macrophages, and fibroblasts.

In fact, a recent study has indicated that insensate diabetic foot ulcers do best in an environment in which they are frequently débrided.³⁴ Applying a growth factor in a liquid or gel might not insure availability to the “wound healing” cells in the wound; however, a few human studies have shown that topical administration of PDGF may favorably augment wound-healing.¹ Indeed, human wound healing studies using all topically applied growth factors have not shown as dramatic an improvement as would have been expected from the animal studies. One can speculate that the lack of improvement is likely to be related to a problem with delivery rather than to an inherent difference between the wound repair mechanisms of humans and other animals.

It is our contention that in order for a cytokine to work effectively in repairing a neuropathic diabetic foot ulcer it needs to be used in a situation where the foot ulcer is being effectively treated and the cytokine needs to be effectively delivered during the appropriate time frame to cells that are involved in wound repair. In other words, a system of cytokine delivery needs to be developed that allows for the continuous application of the cytokine to keratinocytes, fibroblasts, and endothelial cells.

Adenovirus vector

We propose to overcome the challenge of cytokine delivery for the treatment of a diabetic foot ulcer by using an adenovirus vector for gene transfer of PDGF-BB into the cells involved in wound healing. The application of adenoviral mediated gene transfer to the problem of impaired wound healing is a new approach to wound healing. It is a novel application and, potentially, a clinically important application of gene therapy. In other gene therapy applications, limitations of adenoviral mediated gene transfer include the subject's inflammatory response to adenovirus and the limited duration of transgene expression. However, in our preliminary studies these “limitations” have proven beneficial in the setting of wound healing.

We have focused our efforts on adenoviruses instead of other chemical, physical, or biological gene transfer techniques because of several unique features of this system. Adenoviruses infect all human skin cells at more than 95% efficiency in vitro, are nonlytic, and do not induce apparent phenotypic changes in infected cells.⁵⁸ Adenoviruses remain episomal and rarely integrate into the human genome.⁵⁹ Gene expression in human skin grafted to SCID mice and in mouse skin lasts for at least 2 weeks. Adenoviruses have been constructed with deletions of the E1 gene region (the transforming region) and the E3 gene region (the immune modulatory region)

deleted. Thus, the dl7001 adenoviral vector containing growth factor cDNA at the E1 region can usually only replicate in 293 human embryonic kidney cells.⁵⁹ Replication-deficient Ad5 is safe for use in immunodeficient animals.⁶⁰ Thus, replication-deficient adenoviruses appear ideally suited to enhance growth factor gene expression. Their potential limitations, i.e., the induction of a strong antiviral immune response,⁶¹ in fact, may enhance the wound healing inflammatory response. Unlike other potential applications of adenoviral mediated gene therapy, limited duration of high level transgene expression is all that shall be necessary to achieve the desired effect of healing otherwise nonhealing wounds. In addition, the loss of transgene expression allows resolution of inflammation and the wound to proceed to remodeling.

In summary, by inducing PDGF-B gene overexpression of “wound healing cells,” ample quantities of PDGF-BB will be available in the wound bed in diabetic foot ulcer patients. The combination of gene therapy-induced production of PDGF-BB and a limb compression bandage should dramatically augment wound healing in diabetic foot ulcer patients. This phase I study is designed to determine acute safety and to begin to estimate the effect of this therapy.

GENERAL INVESTIGATIONAL PLAN

It is our hypothesis that induction of PDGF-B gene overexpression in cells in the bed of a diabetic insensate foot ulcer will enhance wound healing dramatically. All patients will receive standard care, which is petroleum jelly, gauze, wound debridement, and the use of special shoes and crutches.^{28,34} This hypothesis has not been tested in humans. Therefore, we propose a standard three-six Phase I dose escalation scheme escalating as high as 5×10^8 particles per ulcer of H5.020CMV.*PDGF-b* to determine the maximum tolerated dose (MTD) of intraulcer application of H5.020CMV.*PDGF-b*.

Objectives

The primary objectives of this study are: to evaluate the acute safety of an intraulcer injection of H5.020CMV.*PDGF-b*, both local and systemic, thereby determining the recommended dose of intraulcer H5.020CMV.*PDGF-b*; and to evaluate the biologic feasibility of an intraulcer injection of H5.020CMV.*PDGF-b* in patients with insensate diabetic foot ulcer.

The secondary objectives are to determine if expected changes in clinical wound characteristics and changes in cellular and molecular repair mechanisms are evident after the local secretion of PDGF-BB is induced by H5.020CMV.*PDGF-b* intraulcer injection.

Trial design

This is an open-label, ascending dose, single arm study of H5.020CMV.PDGF-*b* given as an intraulcer injection in patients with a diabetic insensate foot ulcer.

The primary endpoint is to evaluate the safety and determine the MTD (if $\leq 5 \times 10^8$ particles/ulcer) for the intraulcer application of H5.020CMV.PDGF-*b*.

Secondary endpoints will be improvements in clinical wound characteristics and wound cellular characteristics. The clinical wound characteristics to be assessed will include wound size, wound granulation tissue and wound pain. The cellular characteristics to be assessed will include specific stains or probes for adenovirus vector, PDGF-BB, PDGF-AA, PDGF-receptor α and β , MAC-3, Ki-67, CD-31, CD-45, collagen type I, fibronectin, and counts for the total number of macrophages, neutrophils, and eosinophils.

Chemical, biological, and physical characteristics

The PDGF-B gene was obtained by Dr Meenhard Herlyn from Dr B. Westermarck, University Hospital, Uppsala, Sweden. A 1.2 kilobase fragment, a Pst I/Eco RI fragment of the cDNA clone, which encodes the entire open reading frame, was isolated. This gene was then subcloned into the pSL301 transfer plasmid, cut with Not I. The transfer plasmid pSL301 was provided by the Institute for Human Gene Therapy at the University of Pennsylvania. The pSL301 plasmid provides a CMV immediate early CMV promoter and SV40 poly A region.

The selected vector, labeled H5.020CMV PDGF-B, underwent three rounds of plaque purification, ensured by southern blotting and western blots of expressed protein. The final plaques were subjected to protein analyzes to assess the level of PDGF-B protein expression. The selected vector clone was amplified from one plate of 293 cells through successive concentrations of cells to a final level of 200 \times 150mm plates (approximately 2×10^9 cells). A Master Seed Stock of the final lysate was prepared from the infected 200 plates of 293 cells.

Preclinical animal studies

The biologic activity of the adenoviral PDGF vector was confirmed in vitro by two fibroblast proliferation assays.^{62,63} First, fibroblasts transduced using H5.020CMVPDGF-B showed an increase in proliferation. Second, when fibroblasts were exposed to supernatant of WM239A human melanoma cells (which lacks constitutive PDGF-B expression) that had been transfected with the adenovirus containing the PDGF-B gene they were highly stimulated. Secreted PDGF-BB dimer migrated on Western blots in the 30–35 kDa range in the nonreduced form and monomeric PDGF-B at the 15 kDa

when reduced. Serum-free supernatants of transduced WM239A cells maximally stimulated NIH-3T3 mouse fibroblasts (assessed by a 4-hour pulse of 1 μ Ci of [H^3]-thymidine) at a dilution of 1 : 40–1 : 320. The mitogenic activity of culture supernatant from transduced cells was abolished with neutralizing anti-PDGF-B antibody. In contrast, antibodies to the unrelated monocyte chemoattractive protein-1 had no effect. Similarly, supernatants from cultures of cells transduced with a control adenovirus (LacZ) had no mitogenic activity. Based on standard curves of rh-PDGF induced proliferation H5.020CMVPDGF-B transduced cells produced PDGF-BB at 7.5 μ g/ml/ 5×10^5 cells over 72-hour period. These studies confirm that H5.020CMVPDGF-B induces the production of PDGF-BB and that the PDGF-BB produced is identical biologically and biochemically to native and recombinant PDGF-BB.

Additional studies on H5.020CMVPDGF-B showed that it induces fibroblasts to produce matrix by increasing the fibroblast production of collagens and fibronectin. Fibroblasts transfected by H5.020CMVPDGF-B also change their biological phenotype by dramatically upregulating the fibronectin receptor VLA-5. The increased production of fibronectin coupled with the increased VLA-5 receptor permits the fibroblasts to proliferate and anchor independently in a semisolid media. When grown in monolayer the pattern of cell morphology and organization of H5.020CMVPDGF-B induced growth resembles the lack of contact inhibition. This effect of H5.020CMVPDGF-B is primarily due to an increase in fibroblast produced matrix. Thus, the transformed fibroblast phenotype results from changes in matrix formation and matrix receptor expression.

Induction of endogenous PDGF production by H5.020CMVPDGF-B in human skin

In order to assess the biologic activity of our vector in human skin in vivo we used human skin grafted to the backs of RAG-1 mice.⁶² The human skin was injected intradermally with either PDGF/Ad5 or LacZ/Ad5 as a control. Grossly the grafts treated with H5.020CMVPDGF-B became raised and hyperemic within three days. Immunohistochemistry was performed with anti-PDGF antibody to determine the expression of PDGF in control and PDGF/Ad5 treated skin. In controls, injection of LacZ/Ad5 had no effect on PDGF expression. In contrast, skin injected with the H5.020CMVPDGF-B exhibited intense brown immunoperoxidase staining for PDGF throughout the dermis. Interestingly, although keratinocytes do not express PDGF receptors skin injected with PDGF/Ad5 showed marked proliferative changes in the epidermis suggesting that activated fibroblasts produce mitogens for keratino-

cytes. Of note, wounds in the human skin grafted to SCID mice treated with H5.020CMVPDGF-B are much more likely to close than those treated with vector control (LacZ/Ad5).

Effect of H5.020CMVPDGF-B vector on the impaired wound healing of diabetic mice

In order to evaluate the utility of the induction of endogenous PDGF over-expression in wound healing we used a genetically diabetic mouse model of impaired wound healing.⁶³ The homozygous db/db mice are genetically diabetic developing obesity, insulin resistance, severe hyperglycemia (glucose > 400), resembling adult onset diabetes mellitus in which wound healing is markedly delayed. Using heterozygous db/-mice as controls, 6-mm excisional wounds were made on the flanks of each animal. Excisional wounds in homozygous db/db mice were treated either with H5.020CMVPDGF-B or vehicle alone. At 10 days post wounding the H5.020CMVPDGF-B wounds completely healed, but the vehicle treated wounds did not heal. Histology of these wounds confirms the H5.020CMVPDGF-B treated wounds fully healed. The vehicle treated wounds were not healed and, histologically, few signs of re-epithelialization were present on the ulcer bed.

Ischemic rabbit ear model of impaired wound healing

In order to evaluate the ability of H5.020CMVPDGF-B endogenous over-expression of PDGF to overcome severely impaired wound healing we used excisional wounds in ischemic rabbit ears.^{62,64} Two of the three arteries supplying the rabbit ear were ligated to induce ischemia. Six-mm excisional wounds were then created down to and including removal of the perichondrium of the ear cartilage. In the rabbit model there was no wound contraction. For these ischemic wounds to heal they must completely granulate and re-epithelialize from the periphery of the wound. Removal of the underlying perichondrium prevents the bed of the excisional wound from contributing to wound healing. In ischemic excisional ear wounds treated with vehicle alone there was virtually no healing observed in the wounds at 7 days. In marked contrast, wounds treated with 1×10^8 plaque forming units (PFU) injected intradermally showed complete wound healing by 7 days. Wounds treated with LacZ/Ad5 or 5 μ g of PDGF-BB protein were no different than control wounds.

PARTICIPANT CRITERIA

The patient population to be investigated in this study has diabetes mellitus, a foot ulcer, lack protective sensa-

tion in their foot, and have adequate arterial blood flow. These principles were used to develop the inclusion and exclusion criteria.

Number of participants

The total number of subjects entered into the study will be dependent on the number of subjects who develop DLT (dose limiting toxicity) and the number of dose escalations. This trial has a three-six design and four dose levels. Therefore, the number of subjects required for this study could range from 9 to 24.

Duration of treatment

We anticipate being able to enroll 17 patients per year (i.e., 1–2 per month). All subjects at a given dose level must complete all evaluations before escalating to the next level. Therefore, it is anticipated that it could take as long as 34 months to complete this phase I study.

Inclusion criteria

1. Subject must be unable to perceive 10 g of pressure exerted by a Semmes-Weinstein 5.07 monofilament in the peri-ulcer area.^{23,36}
2. Subject must have received appropriate care for at least 6 weeks without improvement in wound size
3. The size of the study wound must be between 1 and less than 10 cm² after it was been sharply débrided per method of Steed et al.,³⁴ as measured by acetate trace and planimetry.
4. The wound must be located on the midfoot or the forefoot (i.e., The mid-arch distal towards toes. This is the area of the foot most efficiently off loaded by the method to be used in this study).
5. For subjects with more than one wound that meet criteria 3 and 4, one wound will be randomly selected and treated with H5.020CMV.PDGF-b.
6. Patient must have a palpable posterior tibial or dorsal pedal pulse of the effected limb and either a transcutaneous oxygen measurement in the affected foot of greater than 30 mmHg or an ankle brachial index ≥ 0.90 .^{23,24,39}
7. WBC $\geq 3500/\text{mm}^3$, platelets $< 1,000,000/\text{mm}^3$, and hemoglobin $> 10.0 \text{ g}\%$.
8. Signed informed consent
9. Subject age must be greater than 18.
10. If of childbearing potential, the subject must agree to use a medically approved barrier method of birth control.

Exclusion criteria

1. Subject with any active cancer other than a nonmelanomatous skin cancer. If cancer is in remission, sub-

jects will be excluded unless the remission has extended for at least 10 years.

2. Subjects with life expectancy of less than 6 months.
3. Liver function tests (alanine transaminase, aspartic transaminase, alkaline phosphatase and bilirubin) greater than 1.5x upper limit of normal for the reference lab.
4. Serum creatinine of greater than 3.0 mg/dl.
5. Hemoglobin A1C > 10%.
6. Patients with intercurrent organ damage or medical problems that will jeopardize their ability to participate in this study or to heal their wound.
7. Pregnant or lactating females. A pregnancy test will be performed on each fertile premenopausal female prior to entry into the study. Treatment may not begin until the result of the pretreatment pregnancy test is ascertained.
8. Any requirement for systemic corticosteroids or immunosuppressives, or history of corticosteroid or immunosuppressive use in the 4 weeks previous to study entry.
9. Evidence of viral hepatitis infection by presence of hepatitis B surface antigen or hepatitis C antibody.
10. Patient refusal to use or inability to successfully use a off loading device.
11. Any concurrent medical illness that be exacerbated by H5.020CMV.PDGF-*b* administration.
12. Chronic osteomyelitis, as determined by plain film, affecting the area of the target ulcer.
13. Uncontrolled infection or cellulitis involving the affected ulcer.

TRIAL DRUG AND TREATMENT

After the subject has been evaluated, the treatment will consist of a single 1 ml intraulcer administration of H5.020CMV.PDGF-*b* in the wound parallel to the wound edge. Equal volumes of H5.020CMV.PDGF-*b* will be injected covering a total path of 4 cm of wound perimeter or the full wound perimeter, whichever is smaller.

Dosing schedule

Subjects will receive only one dose. The dose level administration is summarized in Table 1. This study will use a standard three-six Phase I dose escalation scheme. Therefore, three subjects will be treated at the lowest dose. If zero of three experience DLT, then the dose will be escalated and three new subjects will be treated at the next higher dose. If one of three of the subjects at a dose experience DLT, then three more, for a total of six, will receive that dose. Of the six subjects who receive the lower dose, if only one of six experience DLT then the

Table 1. Dose escalation schedule

Dose level	Number of patients	PFU/ulcer*	Particles/ulcer
1	3–6	1×10^7	5×10^8
3	3–6	5×10^7	2.5×10^9
3	3–6	1×10^8	5×10^9
4	3–6	5×10^8	2.5×10^{10}

*1 PFU = 50 virus particles.

dose will be escalated to the next higher dose. However, if more than one of six experience DLT (i.e., any of the additional subjects), then the MTD will be declared and the next lower dose will be the recommended dose for future trials. This pattern of administration will be repeated for all subsequent dose levels. In the unlikely event that the initial dose is the MTD, then the subsequent doses will be adjusted so that they are one-half log units of PFU less than the initial dose. Furthermore, if, when the MTD is declared, only three subjects have been treated at the next lowest dose, then three additional subjects will be treated to insure that no more than one in six experience DLT before it is declared the recommended dose.

Toxicity

Each patient will be monitored closely for clinically adverse reactions resulting from treatment from H5.020CMV.PDGF-*b*. The toxicity will be graded according to the National Cancer Institute's Common Toxicity Criteria Scale. Patients will be monitored for three broad classes of toxicity. First, it is anticipated that there may be local toxicity from the injection of H5.020CMV.PDGF-*b* including redness, swelling, pain, and increased warmth at the injection site. Second, patients will be closely monitored for the development of clinical symptoms of systemic toxicity, in particular, signs and symptoms suggesting autoimmune disease or allergic reactions. In addition, evidence for adenoviral infection will be evaluated through history and physical examination. Serology for adenovirus will be obtained at baseline, day 28, week 6, and month 3. Clinical signs and symptoms of active adenoviral syndromes include coryza, pharyngitis, tonsillitis, bronchitis, pneumonia, conjunctivitis, or diarrhea. Patients with clinical symptoms suggestive of adenoviral infection will be cultured.

Both local (wound) and systemic toxicity will be graded. Local toxicity will be graded as follows: erythema and induration less than 2 cm from the edge of the wound equals grade 1; erythema and induration greater than 2 cm from the edge of the wound and/or ulcer enlargement of > 15% of the baseline wound area equals grade 2;

painful (> 33% increase in pain score) enlargement of ulcer greater than 25% of the baseline measured wound area equals grade 3; and permanent dysfunction related to local toxicity equals grade 4. First, patients will not be considered to have local DLT unless they have grade 2 toxicity. Second, patients will be closely monitored for the development of systemic toxicity. Systemic toxicity will be graded according to the National Cancer Institute's Common Toxicity Criteria. An isolated increase in ulcer related pain in response to this single intraulcer injection will not be a form of DLT, but will be treated appropriately with an increase in the subject's opiate analgesia.^{65,66}

Systemic DLT is defined as any vector-related grade 2 or greater toxicity on the National Cancer Institute's Common Toxicity Criteria, with the exceptions outlined below. For elevated liver function tests (i.e., alanine transaminase or aspartate transaminase), DLT of these blood chemistries must be a grade 2 for greater than 48 hours. However, any liver function test grade 4 will be considered DLT. Conversion of patients to antinuclear-antibody positivity or rheumatoid factor positivity will be considered Grade 2 toxicity. Any clinical evidence of autoimmune disease is defined as Grade 2 toxicity. For hypertension, DLT will be considered any grade 3 for an individual who has no history of treatment for hypertension and was normotensive before the administration of H5.020CMV.PDGF-b. For an individual currently receiving therapy, DLT will be defined as any individual requiring a new emergent blood pressure lowering agent. The final exception is for fever. DLT for fever will be defined as any grade 2 that is not responsive to 650 mg of acetaminophen administered every 6 hours.

TRIAL METHODS

Once a subject has been identified, the principal investigator will discuss the study with the patient and verbally assess whether the subject could be a candidate for this study based on the inclusion and exclusion criteria. If it appears that they could be a candidate, then they will be asked to sign a consent form. Once the consent has been signed the following pretreatment and baseline evaluations will be conducted:

1. History, physical examination, including documentation of all measurable disease.
2. Height and weight.
3. Laboratory profile: CBC with differential, platelet count, chemistry panel, thyroid function studies (baseline and day 28 only), and urinalysis.
4. Autoimmune tests: Antinuclear antibody (ANA),

erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF).

5. Serologic tests for adenovirus: neutralizing antibody and western blot analyzes for evidence of previous adenovirus infection. The majority of patients will be seropositive. Positive serology will not exclude a patient from participation in the trial.
6. Hepatitis B and C serologic testing.
7. Electrocardiogram (EKG).

Treatment

1. This safety trial will be 28 days long.
2. The primary wound will be locally débrided of fibrin and necrotic debris as described in Steed et al.³⁴ The wound will be traced and photographed. Either 4-mm biopsy or debridement will occur at day 3 and day 28. This will be repeated at day 3 and day 28.
3. The safety of intraulcer injection of H5.020CMV.PDGF-b will be assessed at four dose levels.
4. The total dose of H5.020CMV.PDGF-b will be in 0.1 cc of fluid. This solution has a viscosity similar to saline. The dose will be injected 0.5 cm from the edge of the wound covering 4 cm of total wound perimeter using a 27-gauge tuberculin syringe.
5. Subjects who develop fever or headache may be given 650 mg of acetaminophen by mouth every 4 hours.
6. All subjects will receive a dressing change everyday post administration of H5.020CMV.PDGF-b.
7. Cohorts of up to 6 patients each will be treated at each dose level.
8. All questions to be asked of the subjects are listed in the CRF. No quality of life questionnaires will be used.

Post treatment (acute)

1. Subjects will continue to use the special shoe and crutches. The wound dressing will be changed daily.
2. Tests and evaluations will be performed during each visit according to Table 2.
3. Wound traces and photographs will be obtained weekly.
4. Biopsy specimens will be placed in Michele's medium. The tissue will then be evaluated with immunohistochemical techniques directed at the efficacy and duration of H5.020CMV.PDGF-b transduction. Evaluations will include specific stains or probes for Adenovirus vector, PDGF-BB, PDGF-receptor a and b, MAC-3, Ki-67, CD-31, CD-45, collagen type I, fibronectin, and counts for the total number of macrophages, neutrophils, and eosinophils.
5. The post treatment part of the study will terminate

Table 2. Frequency for obtaining study parameters

Study parameter	Baseline	Day 1**	Days 2 and 3	Day 7	Day 14	Day 21	Day 28	Week 6 and month 3	Month 6 and month 12
History, Physical Exam, and Vital Signs	X	X*	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X		
CBC@	X	X	X	X	X	X	X	X	
Chemistry Panel +, UA	X	X	X	X	X	X	X	X	
Pregnancy Test #	X						X		
Autoimmune Tests	X			X	X	X	X		
EKG	X								
Ulcer Measurements °	X	X	X	X	X	X	X	X	X
Hepatitis Serology	X								
Ulcer Biopsy ^		X		X			X		
Serological Test Adenovirus and Anti-PDGF Antibodies	X						X	X	
Photograph	X	X	X	X	X	X	X		

+ Serum laboratory studies will include Quantitative PCR for PDGF, the transgene, and viral particles, electrolytes, BUN, Cr, ALT, AST, γ GT, Alk Phos, LDH, Billirubin, TP, albumin, Ca, PO₄, adenoviral shedding AFU assay (blood and urine), and thyroid function tests (baseline and day 28 only). No labs drawn on day 2

@ CBC includes differential and platelets

Only women of child-bearing potential

^ Wound biopsy, not done on day 2

° Wound measurement includes length, width, and computer-based planimetry

*During the GCRC admission vital signs will be obtained at baseline, 15 minutes, 6 hours after injection of H5.020CMV.PDGF-b therapy, and then every shift

**Day 1—injection of H5.020CMV.PDGF-b

28 days after the intraulcer injection of H5.020CMV.PDGF-b.

Post treatment surveillance (long term)

1. Post study surveillance of the participants will continue at six weeks, and 3, 6, and 12 months post H5.020CMV.PDGF-b therapy.
2. At the six-week and three-month clinic visit a physical examination, history, wound assessment and query about adverse events will be recorded. In addition blood will be obtained to monitor hematology, clinical chemistries and serology to PDGF-BB.
3. At the six month and 12 month clinic visit a physical examination, history, wound assessment and query about adverse events will be recorded.

CLINICAL AND LABORATORY

During most of this study, the subject will reside at home. They will be encouraged to maintain their normal diet and activity levels compatible with ulcer nonweight bearing. During the 3 days that the subject resides in the General Clinical Research Center they will receive a normal hospital diet and have activity levels compatible with ulcer nonweight bearing.

History and physical examinations

A full history and physical exam will occur at the baseline visit and on day 28. An abbreviated history and physical

examination will occur at days 0, 2, 7, 14, 21, 28; week 6; and months 3, 6, and 12 (see Table 2).

Vital signs

Vital signs (e.g., blood pressure, pulse rate, respiratory rate, temperature, pain assessment) will be obtained at baseline, 15 minutes, and 6 hours after injection of H5.020CMV.PDGF-b therapy. There after vital signs will be performed every shift while the subject is admitted to the General Clinical Research Center, and during every study visit. An assessment of ulcer related pain using a 1–10 scale will be conducted as part of the vital signs.^{65,66} If the pain measurement increases by more than 33%, subjects will receive appropriate medication for their pain.

Safety laboratory test

The investigator will examine subjects on days 1, 2, and 3 with special emphasis on the foot ulcer. A history and physical, CBC, complete chemistry panel, and urinalysis, will be done per Table 2. Biopsies will also be obtained on days 1, 7, and 28. For a full set of tests to be performed (see Table 2).

In addition, as secondary measures of effect, the patient's wound area and wound perimeter will be evaluated at all visits in this study. Comparison of the change in wound size will be estimated by comparing wound size estimates from day 1 and the current wound measurement.

For long-term safety considerations, subjects will be examined during the post-trial surveillance period (week 6; months 3, 6, and 12). A subject's medical records may be requested from the Healthcare providers for those subjects who develop illnesses that may have been as a result of H5.020CMV.PDGF-b.

Research laboratory tests

Prior to the application of H5.020CMV.PDGF-b, the wound will be debrided per Steed et al.³⁴ The biopsy will be obtained when indicated in Table 2 using standard technique. Hemostasis will be obtained using compression. At day 7 post treatment with H5.020CMV.PDGF-b, a second wound biopsy will be obtained. This biopsy will occur in the injection site quadrant that is most distal from the first biopsy site. A final biopsy will occur at day 28. This biopsy will occur midway between the second biopsy and the first biopsy site. Evaluations of biopsy material will include specific stains or probes for Adenovirus vector, PDGF-BB, PDGF-receptor α and β , MAC-3, Ki-67, CD-31, CD-45, collagen type I, fibronectin, and counts for the total number of macrophages, neutrophils, and eosinophils.

RISKS AND BENEFITS OF PARTICIPATION

This is a Phase I study. H5.020CMV.PDGF-b has not previously been used on humans. H5.020CMV.PDGF-b has not been previously used for the treatment of foot ulcers. It is therefore impossible to know all of the risks or benefits, and it is possible that treatment of a wound with H5.020CMV.PDGF-b could cause the wound to deteriorate. However, the virus has been used in humans in other gene therapy experiments and the topical application of the growth factor PDGF was recently approved for use by the FDA for the treatment of individuals with diabetic foot ulcers. The purpose of this study is to determine if this therapy is safe. The risks for the study are due to procedures/tests and from the gene therapy (H5.020CMV.PDGF-b).

Procedures/tests

Because the H5.020CMV.PDGF-b will be injected into the foot ulcer, risks from the injection could include: pain from the intraulcer injection of H5.020CMV.PDGF-b, local but controllable bleeding from the injection sites, cellulitis, and wound pain in wound areas directly involved with the infiltration. The potential exists that the wound may get worse and that cellulitis of the limb might occur.

Standard care with petroleum jelly gauze, wound debridement, and nonweight bearing has been used for many years and are generally considered safe.²⁸ Compli-

cations of this treatment can include: cellulitis, foot and leg pain, ulcer pain, new wounds, enlarging wound, amputation, loss of balance, and contact dermatitis.

Skin biopsies are commonly performed. Potential adverse events included cellulitis, bleeding, scarring, and nonhealing.

Venipuncture is commonly performed. Blood studies will require between 10 and 15 milliliters of blood per study day evaluation. Adverse events include dizziness, bruising, bleeding, phlebitis, and cellulitis.

Gene therapy

This is the first time H5.020CMV.PDGF-b has been injected into human wounds. It is therefore impossible to anticipate all of the adverse reactions that could occur. Topically applied PDGF-BB was recently approved by the FDA for the treatment of foot ulcers in individuals with diabetes. The package insert for topical PDGF-BB (generic name-Becaplermin, trade name-Regranex) states that the frequency of infection, cellulitis, osteomyelitis, mortality, cardiovascular, respiratory, musculoskeletal, and central and peripheral nervous system disorders was similar between those receiving topical PDGF-BB and those who did not receive topical PDGF-BB. Topically applied PDGF-BB was also felt not to change cells such that they could become mitogenic or carcinogenic. Finally those who used topical PDGF-BB did not develop antibodies against PDGF-BB.

H5.020CMV is a type of adenovirus, which is one of the viruses that causes the common cold. As expected, if inhaled, adenovirus can cause the symptoms of a common cold or flu. H5.020CMV or similar adenoviruses have been administered to more than 50 study subjects at the Hospital of the University of Pennsylvania. The side-effects that have been noted are low grade fevers, rash, and abnormalities in liver function tests. These abnormalities may persist for a few days but have always returned to baseline.

It should also be noted that a patient with a metabolic disorder that was enrolled in a gene therapy trial at the University of Pennsylvania and received an adenovirus gene vector via a hepatic vein injection died. This patient's death may have been related to complications of the administration of the adenovirus gene vector.

ADVERSE EVENT REPORTING AND PARTICIPANT WITHDRAWALS

Adverse Event: An adverse event (AE) or adverse drug experience is defined as any medical problem experienced by a study participant that is not a benefit to

the participant whether or not considered intervention-related by the investigators.

Serious Adverse Event: Serious adverse event (SAE) refers to any adverse event or adverse drug experience that occurs at any dose that results in any of the following:

1. Any fatal event
2. Any life threatening event (in the opinion of the investigator, the participant is at immediate threat of death)
3. Any event that causes a significant disability/incapacity (substantial disruption of a person's ability to conduct normal life functions)
4. Any event which requires previously unscheduled inpatient hospitalization or prolongs hospitalization
5. Any event that causes a congenital anomaly or birth defect
6. Any event in the judgment of the physician that does not fulfill a criterion listed above but jeopardizes the patient may be considered an SAE when based on appropriate medical judgment. In addition, any event that may require medical or surgical intervention to prevent one of the outcomes listed in the definition may also be considered an SAE.

Treatment-emergent Adverse Event: A treatment-emergent adverse event is defined as any adverse event not present prior to gene instillation or any event already present that worsens in either intensity or frequency following gene instillation.

Baseline-emergent Adverse Event: A baseline-emergent adverse event is defined as any adverse event which occurs or worsens during the screening process (after informed consent) before gene instillation on day 1.

Eliciting and recording adverse events

Reporting of adverse events will be accomplished by collecting information on adverse experiences during the screening process and at scheduled follow-up visits. In order to avoid bias in eliciting AEs, participants will be asked "Have you had any changes in your health since last visit?" All AEs (serious and nonserious; treatment-emergent and baseline-emergent) must be recorded on the AE Report Form and also the SAE Report Form if applicable.

Sponsor must provide a written report of all serious and unexpected AEs to the FDA within 15 calendar days of the event. If the event is a death or life-threatening and unexpected, the FDA must be notified by telephone or fax within seven calendar days, followed by the written reports within 15 days of the event.

In order to facilitate timely reporting of SAEs to the FDA by the Sponsor, clinical research staff must contact the Sponsor immediately and Fax: the completed AE Report Form and initial SAE Report Form prior to the close of the following business day. It is important to note that all serious and unexpected AEs must be reported to the Sponsor, regardless of the intervention-related assessment. In addition, the Principal Investigator is responsible for complying with local Institution's IRB regulations regarding SAE reporting.

When reporting fatal, life-threatening, or serious and unexpected AEs, the research center personnel should be prepared to provide as much of the following information as is available at the time:

1. Subject initials and identification number
2. Investigator and primary physician's name (if different)
3. Protocol title and number
4. Subject's date of birth, sex, and ethnicity
5. Total number of gene instillations
6. Concurrent medications, including dose, route of administration, and duration of therapy
7. Information regarding the event, including the following details:

Description

Date of onset and end date (if appropriate)

Whether hospitalization or prolonged hospitalization was required

Treatment required for the AE

Treatment outcome

Investigator's determination of relationship to the gene instillation

Whether the AE is life-threatening

Expedited reports

AEs are recorded on the FDA-style AE case report form. The form is continuously updated as events happen. The AE form is faxed to the Sponsor in no later than 48 hours after the onset of the event.

Subject withdrawal

Subjects will be withdrawn from the study for any of the following reasons:

1. Subject wishes to withdraw from the trial.
2. Subject develops serious concurrent illness unrelated to diabetic foot ulcer.
3. Subject is not compliant with study rules and procedures (e.g., attendance at visits within the appropriate time frame).

PARTICIPANT RECRUITMENT AND RETENTION

Study subjects will be recruited from patients seen at the Cutaneous Ulcer Center at the University of Pennsylvania and from the practices of other participating physicians who work at the University of Pennsylvania. It is anticipated that 15 patients per year will be recruited for this study. All subjects will be given an IRB approved consent form. The study will be discussed with them in detail prior to their agreeing to participate in the study. The principal investigator will also inform them that their participation is voluntary. If they decide not to participate their decision will not affect their current care or relationship with their health care provider.

Formally to enter a recruited patient, the investigator will contact the study nurse or the Sponsor. The following information will be requested:

1. Patient name and study number.
2. All patient eligibility requirements will be verified.
3. Signed informed consent.
4. Confirmation of dose level assignment.

Participant retention

The subject is not required to enter this study. Their participation is voluntary and they may withdraw from this study at any time. Withdrawal from this study will not prejudice present or future care by the investigators, collaborators, or any other health care provider at the University of Pennsylvania Medical Center.

Since this a novel treatment, subjects will be closely monitored initially as an inpatient and then as an outpatient. Since subjects with diabetic foot ulcers are very infrequently hospitalized, this close follow-up could be viewed as a barrier to participation. Furthermore, the inclusion and exclusion criteria will limit subject participation to those with wounds that are unlikely to heal. Wounds of this size range comprise about 40% of all individuals with diabetic foot ulcers.

Financial considerations

There will be no cost to the subject for care related to the study. In the event of an injury resulting from research procedures, the cost for medical treatment, in excess of that covered by a third party payer (insurance) will be provided without cost to the subject by the Hospital of the University of Pennsylvania, but financial compensation for injury is not available.

Subjects who complete the full study will receive a stipend of 100 dollars to help defray the costs incurred for their participation in the study (e.g., transportation to the study site and parking at the study site). Subjects

will receive this compensation within two months after full completion of the study.

STATISTICAL CONSIDERATIONS

The MTD for this trial will be defined as the highest dose that is equal to or less than 5×10^8 particles per ulcer of H5.020CMV.PDGF-*b* in which zero or one of six subjects demonstrates DLT as defined in section 5.3. It is recognized, as has been true in other studies using adenovirus vectors that the MTD may not be reached in this trial at the range of doses proposed. In this case, the highest dose of 5×10^8 particles per ulcer will be recommended for future trials.

This study will use a standard three-six dose escalation scheme. For example, three subjects will be treated at the dose level 1. If zero of three experience DLT, then the dose will be escalated and three new subjects will be treated at the next higher dose. If one of three of the subjects at dose level 2 experience DLT, then three more for a total of six will receive the lowest dose. Of the six subjects who receive the dose level 2, if only one of six experience DLT, then the dose will be escalated to the dose level 3. However, if more than one of six experience DLT (i.e., any of the additional subjects or two of the original three at dose level 2) then the MTD will be declared as dose level 1 and this dose will be used in future trials. This pattern of administration will be repeated for all subsequent dose levels until MTD is declared. In the unlikely event that more than two subjects experience DLT at dose level 1, then the subsequent doses will be adjusted so that they are one-half log units of PFU less than dose level 1. Furthermore, if when 2 subjects experience DLT, only three subjects had been treated at the next lowest dose, then three additional subjects will be treated to insure that no more than one in six experience DLT before it is declared the MTD or recommended dose.

The operating characteristics of this design are shown in Table 3. This table provides the probability of escalation to the next highest dose for each underlying true DLT rate. For example, for a toxicity that occurs in 10% of subjects, there is a greater than 90% chance probability of escalating. Conversely, for a common toxicity, which occurs at a rate of 70%, the probability of escalating is less than 5%. Another important consideration is the probability of failing to observe a toxicity in a Phase I study using a sample size design of three-six. This is shown in Table 4. For example, with six patients, the probability of failing to observe toxicity, which occur at least 40% of the time is less than 5%.

Table 3. True underlying DLT rate at a given dose level

	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of dose escalation	0.91	0.71	0.49	0.31	0.17	0.08	0.03	0.01	0.001

Table 4. Probability of failing to observe DLT

n	10%	20%	30%	40%	50%	60%	70%	80%	90%
3	0.73	0.51	0.34	0.22	0.13	0.064	0.027	0.008	0.001
4	0.53	0.26	0.12	0.047	0.004	<0.001	<0.001	<0.001	<0.001

Secondary endpoints

This sample size determination for this study is dependent on the primary endpoint and is described above. The secondary endpoints will be used to help establish proof of concept. For example, if *H5.020CMV.PDGF-b* does stimulate wound healing cells to secrete PDGF-BB then a local measurable increase in PDGF-BB should be shown. Statistical analyzes for these endpoints will be primarily descriptive and will include summary statistics such as means, medians, and proportions, when appropriate.

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REFERENCES

- Weiman TJ, Griffiths GD, Polk HC. Management of diabetic mid-foot ulcers. *Ann Surg* 1992;215:627–32.
- National Diabetes Data Group. Diabetes in America, 2. NIH Publication no. 95–1468. National Institute of Health, 1995:1–782.
- LEA study group. Comparing the incidence of lower extremity amputations across the world: the global lower extremity amputation study. *Diabetic Med* 1995;12:14–8.
- Ramsey SD, Newton K, Blough D, McCulloch DK, Sandu N, Reiber GE, Wagner EH. Incidence, outcomes, and cost of foot ulcers in patients with diabetes. *Diabetes Care* 1999;22:382–7.
- Reiber GE. The epidemiology of diabetic foot problems. *Diabetic Med* 1998;13:S6–S11.
- McNeely MJ, Boyko EJ, Ahroni JH, Stensel VL, Reiber GE, Smith DG, Pecoraro RF. The independent contributions of diabetic neuropathy and vasculopathy in foot ulceration. *Diabetes Care* 1995; 18:216–9.
- Valway SE, Linkins RW, Gordes DM. Epidemiology of lower-extremity amputations in the Indian health service. 1982–87. *Diabetes Care* 1993;16:349–53.
- Adler AI, Boyko EJ, Ahroni JH, Smith DG. Lower-extremity amputation in diabetes. The independent effects of peripheral vascular disease, sensory neuropathy, and foot ulcers. *Diabetes Care* 1999; 22:1029–35.
- Jeffcoate WJ, Macfarlane RM, Fletcher EM. The description and classification of diabetic foot lesions. *Diabetic Med* 1993;10:676–9.
- Connor H. Diabetic foot disease—where is the evidence? *Diabetic Med* 1999;16:799–800.
- Phillips TJ. Chronic cutaneous ulcers. Etiology and epidemiology. *J Invest Dermatol* 1994;102:S38–S41.
- Lindholm C, Bjellerup M, Christensen OB, Zederfeldt B. Quality of life in chronic leg ulcer patients. An assessment according to the Nottingham Health Profile. *Acta Dermato-Venereol* 1993;73: 440–3.
- Shaw JE, Zimmet PZ. The epidemiology of diabetic neuropathy. *Diabetes Rev* 1999;7:245–52.
- Larsson J, Agardh CD, Apelqvist J, Stenstrom A. Long-term prognosis after healed amputation in patients with diabetes. *Clin Orth Rel Res* 1998;350:149–58.
- Brod M. Quality of life issues in patients with diabetes and lower extremity ulcers: patients and care givers. *Quality Life Res* 1998; 7:365–72.
- Apelqvist J. Wound healing in diabetes. Outcome and costs. *Clin Pod Med Surg* 1998;15:21–39.
- Ashry HR, Lavery LA, Armstrong DG, Lavery DC, van Houtum, WH. Cost of diabetes-related amputations in minorities. *J Foot Ankle Surg* 1998;37:186–90.
- Gentzkow GD, Iwasaki SD, Hershon KS, Mengel M, Prendergast JJ, Ricotta JJ, Steed DP, Lipkin S. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 1996; 19:350–4.
- Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. *Diabetes Care* 1998;21:822–7.
- Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. *J Vascular Surgery* 1995;21: 71–8.
- Steed DL, Goslen JB, Holloway GA, Malone JM, Bunt TJ, Webster MW. Randomized prospective double-blinded trial in healing chronic foot ulcers. *Diabetes Care* 1992;15:1598–604.
- Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999;22:157–62.
- Pecoraro RE, Ahroni JH, Boyko EJ, Stensel VL. Chronology and determinants of tissue repair in diabetic lower-extremity ulcers. *Diabetes* 1991;40:1305–13.
- Reiber GE, Pecoraro RE, Koepsell TD. Risk factors for amputation in patients with diabetes mellitus: a case control study. *Ann Intern Med* 1992;117:97–105.

25. Reiber GE. The epidemiology of diabetic foot problems. *Diabetic Med* 1998;13:S6-S11.
26. Valway SE, Linkins RW, Gordes DM. Epidemiology of lower-extremity amputations in the Indian Health Service, 1982-87. *Diabetes Care* 1993;16:349-53.
27. Apelqvist J, Larsson J, Agardh CD. Medical risk factors in diabetic patients with foot ulcers and severe peripheral vascular disease and their influence on outcome. *J Diabet Comp* 1992;6:167-74.
28. American Diabetes Association. Consensus development conference on diabetic wound care. *Diabetes Care* 1999;22:1354-60.
29. Tannenbaum GA, Pomposelli FB, Marcaccio EJ, Gibbons GW, Campbell DR, Freeman DV, Miller A, LoGerto FW. Safety of vein bypass grafting to the dorsal pedal artery in diabetic patients with foot infections. *J Vasc Surg* 1992;15:982-90.
30. Shortell CK, Ouriel K, DeWeese JA, Green RM. Peroneal artery bypass: a multifactorial analysis. *Ann Vasc Surg* 1992;6:15-9.
31. Spence LD, Hartnell GG, Reinking G, Gibbons G, Pomposelli F, Clouse ME. Diabetic versus nondiabetic limb-threatening ischemia: outcome of percutaneous iliac intervention. *Am J Roentgenol* 1999;172:1335-41.
32. Van Gils CC, Wheeler LA, Mellstrom M, Brinton EA, Mason S, Wheeler CG. Amputation and prevention by vascular surgery and podiatry collaboration in high-risk diabetic and nondiabetic patients. *Diabetes Care* 1999;22:678-83.
33. Steed DL. Foundations of good ulcer care. *Am J Surg* 1998;176 (Suppl 2A):20S-25S.
34. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg* 1996;183:61-4.
35. Doucette MM, Fyelling C, Knighton DR. Amputation prevention in a high-risk population through comprehensive wound-healing protocol. *Arch Phys Med Rehab* 1989;70:780-5.
36. Flynn MD, Tooke JE. Aetiology of the diabetic foot ulceration: a role for the microcirculation? *Diabetic Med* 1992;8:320-9.
37. Grunfeld C. Diabetic Foot Ulcers: etiology, treatment, and prevention. *Adv Int Med* 1991;37:103-32.
38. Boyko EJ, Ahroni JH, Stensel V, Forsberg RC, Davignon DR, Smith DG. A prospective study of risk factors for diabetic foot ulcer. The Seattle Diabetic Foot Study. *Diabetes Care* 1999;22:1036-42.
39. Boyko EJ, Ahroni JH, Stensel VL, Smith DG, Davignon DR, Pecoraro RE. Predictors of transcutaneous oxygen tension in the lower limbs of diabetic subjects. *Diabetic Med* 1995;13:549-54.
40. Boulton AJ, Malik RA. Diabetic neuropathy. *Med Clin N Am* 1998;82:909-29.
41. Boulton AJM, Gries FA, Jervell JA. Guidelines for the diagnosis and outpatient management of diabetic peripheral neuropathy. *Diabetes Rev* 1999;7:237-44.
42. Wunderlich RP, Armstrong DG, Husain K, Lavery LA. Defining loss of protective sensation in the diabetic foot. *Adv Wound Care* 1998;11:123-8.
43. Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment: a meta analysis. *Diabetes Care* 1999;22:692-5.
44. Falanga V, Margolis D, Alvarez O, Auletta M, Maggiasimo F, Altman M, Jensen J, Sabolinski M, Hardin-Young J. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. *Arch Derm* 1998;134:293-300.
45. Margolis DJ, Lewis VL. A literature assessment of the use of miscellaneous topical agents, growth factors, and skin equivalents for the treatment of pressure ulcers. *Dermatol Surg* 1995;21:145-8.
46. Lundeberg TCM, Eriksson SV, Malm M. Electrical nerve stimulation improves healing of diabetic ulcers. *Ann Plast Surg* 1992;29:328-31.
47. Myerson M, Papa J, Eaton K, Wilson K. The total contact cast for the management of neuropathic plantar ulceration of the foot. *J Bone Joint Surg* 1992;74-A:261-9.
48. Margolis DJ, Cohen JH. Management of chronic venous leg ulcers: a literature-guided approach. *Clin Dermatol* 1994;12:19-26.
49. Fletcher A, Cullum F, Sheldon TA. A systematic review of compression treatment for venous leg ulcers. *BMJ* 1997;315:576-80.
50. Cheatele TR, Scurr JH, Coleridge Smith PD. Drug treatment of chronic venous insufficiency and venous ulceration: a review. *J R Soc Med* 1991;84:354-8.
51. Clark RAF. Wound repair: Overview and general considerations. In: Clark RAF, editor. *The molecular and cellular biology of wound repair*. New York: Plenum, 1996: 3-50.
52. Martin P. Wound healing—Aiming for perfect skin regeneration. *Science* 1997;276 (5309):75-81.
53. Danilenko DM, Ring BD, Tarpley JE, Van Morris BGY, Morawiecki A, Callahan W, Goldenberg M, Hershenson S, Pierce GF. Growth factors in porcine full and partial thickness burn repair. Differing targets and effects of keratinocyte growth factor, platelet-derived growth factor-BB, epidermal growth factor, and neu differentiation factor. *Am J Pathol* 1995;147:1261-77.
54. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878-81.
55. Pierce GF, Mustoe TA. Pharmacologic enhancement of wound healing. *Ann Rev Med* 1995;46:467-81.
56. Pierce GF, Tarpley JE, Tseng J, Bready J, Chang D, Kenney WC, Rudolph R, Robson MC, Vande Berg J, Reid P. Detection of platelet-derived growth factor (PDGF) -AA in actively healing human wounds treated with recombinant PDGF-BB and absence of PDGF in chronic nonhealing wounds. *J Clin Invest* 1995;96:1336-50.
57. Beer HD, Longaker MT, Werner S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. *J Invest Dermatol* 1997;109:132-8.
58. Mulligan RC. The basic science of gene therapy. *Science* 1993;260:929-32.
59. Bett AJ, Prevec L, Graham FL. Packaging capacity and stability of human adenovirus type 5 vectors. *J Virol* 1993;67:5911-21.
60. Kozarsky KF, Wilson JM. Gene therapy: adenovirus vectors. *Curr Opin Gene Dev* 1993;3:499-503.
61. Kass-Eisler A, Falck-Pedersen E, Elfenbein DH, Alvira M, Buttrick PM, Leinwand LA. The impact of developmental stage, route of administration and the immune system on adenovirus-mediated gene transfer. *Gene Ther* 1992;1:395-403.
62. Liechty KW, Sablich TJ, Adzick NS, Crombleholme TM. Recombinant adenoviral mediated gene transfer in ischemic impaired wound healing. *Wound Rep Reg* 1999;7:148-53.
63. Sylvester KG, Nesbit M, Radu A, Herlyn M, Adzick NS, Crombleholme TM. Adenoviral-mediated gene transfer in wound healing: acute inflammatory response in human skin in the SCID mouse model. *Wound Rep Reg* 2000;8:36-44.
64. Liechty KW, Nesbit M, Herlyn M, Radu A, Adzick NS, Crombleholme TM. Adenoviral-mediated overexpression of platelet-derived growth factor-B corrects ischemic impaired wound healing. *J Invest Dermatol* 1999;113:375-83.
65. Max MB, Lynch SA, Muir J, Shoaf SE, Smoller B, Dubner R. Effects of desipramine, amitriptyline, and fluoxetine on pain in diabetic neuropathy. *N Engl J Med* 1992;326:1250-6.
66. Jacox A, Carr DB, Payne R. Management of cancer pain. 1994. Agency for Health Care Policy and Research.