SUMMARY OF SAFETY AND EFFECTIVENESS DATA

GENERAL INFORMATION

Device Generic Name:	Interactive Wound Dressing
Device Trade Name:	DERMAGRAFT®
Applicant's Name and Address:	Advanced Tissue Sciences, Inc. 10933 North Torrey Pines Road La Jolla, California 92037-1005
Premarket Approval Application (PMA):	P000036
Date of Panel Recommendation:	Not applicable
Date of Notice of Approval of Application:	September 28, 2001

INTENDED USE/INDICATIONS

DERMAGRAFT® is indicated for use for the treatment of full-thickness diabetic foot ulcers greater than six weeks duration which extend through the dermis, but without tendon, muscle, joint capsule or bone exposure. DERMAGRAFT® should be used in conjunction with standard wound care regimens and in patients that have adequate blood supply to the involved foot.

DEVICE DESCRIPTION

DERMAGRAFT® is a cryopreserved human fibroblast-derived dermal substitute; it is composed of fibroblasts, extracellular matrix, and a bioabsorbable scaffold. DERMAGRAFT is manufactured from human fibroblast cells derived from newborn foreskin tissue. During the manufacturing process, the human fibroblasts are seeded onto a bioabsorbable polyglactin mesh scaffold. The fibroblasts proliferate to fill the interstices of this scaffold and secrete human dermal collagen, matrix proteins, growth factors and cytokines, to create a three-dimensional human dermal substitute containing metabolically active, living cells. DERMAGRAFT does not contain macrophages, lymphocytes, blood vessels, or hair follicles.

The human fibroblast cells are from a qualified cell bank, which has been extensively tested for animal viruses, retroviruses, cell morphology, karyology, isoenzymes, and tumorigenicity. Reagents used in the manufacture of DERMAGRAFT are tested and found free from viruses, retroviruses, endotoxins,

and mycoplasma before use. DERMAGRAFT is manufactured with sterile components under aseptic conditions within the final package. Prior to release for use, each lot of DERMAGRAFT must pass USP Sterility (14-day), endotoxin, and mycoplasma tests. In addition, each lot meets release specifications for collagen content, DNA, and cell viability. Maternal blood sera is tested for evidence of infection with human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), hepatitis B virus, (HBV), hepatitis C virus (HCV), syphilis, human T-lymphotropic virus type 1 (HTLV-1), and found negative for the purposes of donor selection. During subsequent screening of the fibroblast cell strain at various stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus (EBV) and human T-lymphotropic virus type 2 (HTLV-2), is carried out and found to be negative.

DERMAGRAFT is supplied frozen in a clear bag containing one piece of approximately 2 in x 3 in (5 cm x 7.5 cm) for a single-use application.

IV. CONTRAINDICTIONS

- DERMAGRAFT is contraindicated for use in ulcers that have signs of clinical infection or in ulcers with sinus tracts.
- DERMAGRAFT is contraindicated for use in patients with known hypersensitivity to bovine products, as it may contain trace amounts of bovine proteins from the manufacturing medium and storage solution.

The warnings and precautions can be found in the DERMAGRAFT labeling, which is available on the FDA web site at *http://www.fda.gov/CDRH/pdf/p000036.pdf*.

V. ALTERNATIVE PRACTICES AND PROCEDURES

Alternative therapies include debridement of necrotic tissue, which can significantly reduce bacterial colonization of the ulcer, and saline moistened dressings. Excessive pressure at the ulcer site is alleviated with the use of special pressure relieving inserts or orthotics. Decisions of what treatments are necessary for ulcers are affected by the results of a vascular evaluation of the patient. If vascular supply is insufficient, surgical procedures may be necessary, including arterial bypass grafting. If the vascular supply is sufficient, the most common therapy for ulcers is debridement and wound dressing, as described above.

VI. POTENTIAL ADVERSE EFFECTS

A total of 695 patients were evaluated in four clinical trials, 389 treated with DERMAGRAFT, and 306 treated with Control. Adverse events that were reported in the pivotal 314-patient clinical trial at a frequency of greater than 1% for patients treated with DERMAGRAFT are presented in Table 1. Adverse Event data are also presented, combined, from three previous studies.

Table 1
Adverse Events Reported in Greater than 1% of Patients Treated with DERMAGRAFT

Event	Pivotal Study		Previous Studies	
	DERMAGRAFT N = 163 n (%)	Control N = 151 n (%)	DERMAGRAFT N = 226 n (%)	Control N = 155 n (%)
Infection (study wound)[1]	17 (10.4)	27 (17.9)	63 (27.9)	43 (27.7)
Infection (non-study wound)	17 (10.4)	14 (9.3)	33 (14.6)	22 (14.2)
Accidental injury[2]	17 (10.4)	18 (11.9)	17 (7.5)	11 (7.1)
Skin dysfunction/Blister	16 (9.8)	20 (13.2)	38 (16.8)	31 (20.0)
Flu syndrome	15 (9.2)	9 (6.0)	7 (3.1)	8 (5.2)
Osteomyelitis (study wound)	14 (8.6)	13 (8.6)	17 (7.5)	8 (5.2)
Surgeries involving study ulcer[3]	13 (8.0)	21 (13.9)	35 (15.5)	13 (8.4)
Wound enlargement/Skin ulcer (non-study wound)	12 (4.0)	17 (11.3)	30 (13.3)	16 (10.3)
Cellulitis (study wound)	12 (7.4)	14 (9.3)	25 (11.1)	10 (6.5)
Cellulitis (non-study wound)	10 (6.1)	7 (4.6)	15 (6.6)	13 (8.4)
Peripheral edema/Localized swelling	9 (5.5)	7 (4.6)	20 (8.8)	6 (3.9)
Pharyngitis/URI	7 (4.3)	5 (3.3)	13 (5.8)	11 (7.1)
Pain	6 (3.7)	5 (3.3)	24 (10.6)	12 (7.7)
Lab test abnormal-chemistry[4]	6 (3.7)	5 (3.3)	37 (16.4)	31 (20.0)
Skin disorder[5]	5 (3.1)	4 (2.6)	0 (0.0)	0 (0.0)
Osteomyelitis (non-study wound)	5 (3.1)	2 (1.3)	10 (4.4)	6 (3.9)
Wound enlargement/Skin ulcer (study wound)	4 (2.5)	8 (5.3)	12 (5.3)	15 (9.7)
Urinary tract infection	4 (2.5)	1 (0.7)	7 (3.1)	6 (3.9)
Diarrhea	4 (2.5)	5 (3.3)	4 (1.8)	3 (1.9)
Rash	3 (1.8)	2 (1.3)	4 (1.8)	4 (2.6)
Myocardial infarct	3 (1.8)	0 (0.0)	0 (0.0)	4 (2.6)
Fever	3 (1.8)	0 (0.0)	8 (3.5)	3 (1.9)
Allergic reaction	3 (1.8)	1 (0.7)	1 (0.4)	1 (0.6)
Rhinitis	2 (1.2)	1 (0.7)	2 (0.9)	2 (1.3)
Nail disorder	2 (1.2)	3 (2.0)	1 (0.4)	3 (1.9)
Myalgia	2 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Joint disorder	2 (1.2)	1 (0.7)	1 (0.4)	0 (0.0)
Headache	2 (1.2)	1 (0.7)	3 (1.3)	3 (1.9)
Gastrointestinal disorder	2 (1.2)	3 (2.0)	0 (0.0)	1 (0.6)
Chest pain	2 (1.2)	1 (0.7)	4 (1.8)	5 (3.2)
Anemia	2 (1.2)	0 (0.0)	4 (1.8)	0 (0.0)
Bronchitis	1 (0.6)	1 (0.7)	7 (3.1)	1 (0.6)
Eccymosis	1 (0.6)	0 (0.0)	5 (2.2)	0 (0.0)
Sinusitis	1 (0.6)	0 (0.0)	4 (1.8)	3 (1.9)
Neuropathy	1 (0.6)	0 (0.0)	4 (1.8)	0 (0.0)
Nausea	1 (0.6)	2 (1.3)	4 (1.8)	1 (0.6)
Dyspnea	1 (0.6)	1 (0.7)	4 (1.8)	0 (0.0)
Vomiting	1 (0.6)	1 (0.7)	3 (1.3)	2 (1.3)
Sepsis/Septicemia	1 (0.6)	1 (0.7)	3 (1.3)	0 (0.0)
Gastroenteritis	1 (0.6)	0 (0.0)	3 (1.3)	1 (0.6)
Chills	1 (0.6)	0 (0.0)	3 (1.3)	3 (1.9)
Cataract	1 (0.6)	0 (0.0)	3 (1.3)	1 (0.6)
Angina pectoris	1 (0.6)	0 (0.0)	3 (1.3)	3 (1.9)
Wound drainage	0 (0.0)	0 (0.0)	11 (4.9)	5 (3.2)
Cerebrovascular accident	0 (0.0)	0 (0.0)	7 (3.1)	1 (0.6)
Congestive heart failure	0 (0.0)	3 (2.0)	6 (2.7)	1 (0.6)
Cough increased	0 (0.0)	2 (1.3)	5 (2.2)	2 (1.3)
Back pain	0 (0.0)	1 (0.7)	5 (2.2)	4 (2.6)
Peripheral vascular disorder	0 (0.0)	0 (0.0)	4 (1.8)	0 (0.0)
Retinal disorder/Retinopathy	0 (0.0)	0 (0.0)	3 (1.3)	1 (0.6)
Neoplasm[5]	0 (0.0)	0 (0.0)	3 (1.3)	1 (0.6)
Lab test abnormal – urinalysis	0 (0.0)	0 (0.0)	3 (1.3)	2 (1.3)
Cyst	0 (0.0)	0 (0.0)	3 (1.3)	1 (0.6)
Asthenia	0 (0.0)	0 (0.0)	3 (1.3)	0 (0.0)

[1] Infections include all local wound infections, regardless of etiology (e.g. bacterial, fungal), not including osteomyelitis and cellulitis.

[2] Examples of verbatim codes included in this category are: laceration, foreign body in eye, head injury, dislocation of hip, coccyx fracture post fall, skin tear, and burn right index finger.

[3] Surgical procedures to the study ulcer are defined as any procedure (i.e., surgical debridement more extensive than required by protocol, incision and drainage, revision, excision, or amputation) that occurred during the course of the study.

[4] Pilot study codes to “Lab Tests Abnormal” and does not distinguish between Chemistry, Hematology, and Urinalysis.

[5] None of the events reported under “Skin disorder” involved the study ulcer. Under “Neoplasm”, none of the events reported involved the study leg for the DERMAGRAFT-treated patients.

MARKETING HISTORY

DERMAGRAFT has only been available in the United States as an investigational device under IDE G900155. DERMAGRAFT for the treatment of diabetic foot ulcers was approved for sale in Canada in August 1997. DERMAGRAFT was introduced in the United Kingdom in October 1997, and several other European countries, New Zealand and Australia. As of this submission, the device is available for commercial distribution in Australia, Canada, Finland, France, Hong Kong, Ireland, The Netherlands, New Zealand, Singapore, and The United Kingdom.

SUMMARY OF PRE-CLINICAL STUDIES

This section provides summaries of preclinical tests performed on DERMAGRAFT. Table 2 describes the non-clinical laboratory studies and has been divided into the following categories: Development and Characterization, Immunology, and Toxicology studies. The studies reported here include a range of topics assessing safety, device attributes, practical aspects of device delivery, and potential clinical issues.

Table 2
Pre-clinical Studies with DERMAGRAFT

Study	Results/Conclusions
Development and Characterization	
Assessment of the in vitro aging of fibroblasts in DERMAGRAFT	No significant loss of proliferative capacity before 45 doublings was observed and cells continued to grow for approximately 60 doublings. Fibroblasts used in the manufacture of DERMAGRAFT are within a region of the cultural life span in which synthetic and proliferative activities may be expected to be consistent.
Evaluation of DERMAGRAFT cell banks for the presence of other cell types	Flow cytometric analyses (fluorescence activated cell sorting [FACS]) did not detect the presence of keratinocytes in the fibroblast cell banks.
Feasibility Study - Grafting of a Cultured Skin Equivalent grown on mesh framework (Nylon) into full thickness wounds in Rats	Results showed incorporation of the grafts into the wounds and complete epithelialization. Histologic examination revealed keratinocytes, fibroblasts, collagen, adipocytes and smooth muscle fibers arranged in a natural configuration around nylon mesh fibers.
DERMAGRAFT as a Dermal Replacement in Mini-Pigs to determine if the device cultured with human fibroblasts would incorporate safely into full thickness wounds.	Evidence of the safety and biocompatability of a device cultured with human fibroblasts was established in a porcine model with implants over six months.
Athymic Mouse Studies to support DERMAGRAFT in the treatment of full thickness burn wounds and evaluate the viability of DERMAGRAFT with respect to wound healing	Evaluated the ability of the device to support healing and re-epithelialization of meshed, split-thickness human cadaver skin placed on surgically excised wounds in mice. Percent epithelialization (healing) via histological evaluation, graft take and vascularization rated on data sheets and sent to outside agency for statistical analysis.

Development and Characterization (cont'd.)	
Stimulation of Angiogenesis in the Chick Chorioallantoic Membrane (CAM) assay	Three dimensional fibroblast cultures induced vessel development in the CAM to a greater extent than control. Capillary development is characteristic of VEGF induced angiogenesis. Preincubation of the fibroblast culture with anti-VEGF neutralizing antibody reduced the angiogenic activity to control values.
Angiogenesis in the Aortic Ring Assay	Aorta rings co-cultured with DERMAGRAFT demonstrated a significant increase in the number of microvessels formed ($p=0.028$).
Stimulation [^{3}H]-thymidine incorporation in cultures of Human Umbilical Vein Endothelial Cells (HUVEC)	Medium conditioned from three dimensional fibroblast cultures stimulated [^{3}H]-thymidine incorporation in cultures of HUVEC. The stimulation of [^{3}H]-thymidine incorporation appeared to be dose dependent.
Stimulation of Endothelial Cell Chemokinesis	Results demonstrate DERMAGRAFT stimulates endothelial cell migration.
Stimulation of Endothelial Cell Chemotaxis	Medium conditioned by DERMAGRAFT stimulated HUVEC migration.
Inhibition of Endothelial Cell Apoptosis	Microvascular endothelial cells when co-cultured with DERMAGRAFT continued to proliferate and did not undergo apoptosis.
Expression of Angiogenic Growth Factors	VEGF and HGF mRNA was expressed in DERMAGRAFT. Also detected were PDGF A-chain, TGFβ_1,G-CSF and angiopoietin I.
Splice Variants of VEGF in Fibroblasts	Splice variants of VEGF corresponding to the diffusable and extracellular matrix-binding forms of VEGF were detected in RNA isolated from DERMAGRAFT via PCR technique.
Induction of Integrin $\alpha_v\beta_3$	Medium conditioned by DERMAGRAFT stimulated a significant increase in the expression of Integrin $\alpha_v\beta_3$ in HUVEC.
Detection of Growth Factors by ELISA	VEGF, HGF, TGFβ_1, and G-CSF were detected in pg to low ng concentrations by ELISA in medium conditioned by incubation with DERMAGRAFT.
Bovine Serum Albumin (BSA) Content of DERMAGRAFT Rinsing Study	Quantification of the residual amounts of manufacturing materials after the device is thawed and rinsed according to the Directions For Use (DFU). Using a Fluorescent Enzyme Linked Immuno-Assay (FELISA) specific for BSA, 4.6 µg/2"x3" device and 9.1 µg/4"x6" device residual BSA was detected.
Tearing Strength Properties of DERMAGRAFT	Evaluated the critical tearing force of mesh substrate and DERMAGRAFT at various times in culture and freezing at -70°C and -140°C. The time period in which mesh substrate, seeded with fibroblasts, maintains a degree of tear strength and structural integrity is two to three weeks. Components of the mesh substrate maintain integrity during product storage.
Effects of Sterilization on the tensile properties of the mesh substrate.	Quantified the changes in tensile properties of mesh substrate subjected to different radiation sterilization methods E-beam and Gamma radiation at low (3.0) and high doses (6.0 mrad) and to EtO sterilization. Mesh substrate should maintain the same level of mechanical strength throughout the DERMAGRAFT manufacturing process regardless of sterilization process.

Immunology	
Persistence	PCR testing of biopsies in venous ulcers detected DERMAGRAFT cells in human patients up to 6 months after implantation.
Response of DERMAGRAFT to γ-interferon	In scaffold-based (DERMAGRAFT) three dimensional fibroblast cell cultures, γ-interferon caused little induction of CD40 and HLA-DR antigens whereas all cells upregulated the expression of HLA I antigen. Fibroblast cells grown on collagen gel or in monolayer culture, in contrast, upregulated both the expression of HLA I, HLA II and CD40 antigens in response to γ-interferon.
Immunogenicity	Immunogenicity via histological assessment displayed no findings suggestive of an immune response; The potential for DERMAGRAFT to elicit an immune response was evaluated by examining the baseline and terminal sera of patients enrolled in a clinical trial for DERMAGRAFT using Western Blot technique. No clinically significant antibody response to DERMAGRAFT was observed in patients treated with up to 8 pieces of DERMAGRAFT.
Toxicology Studies (sterilized plastics and polymeric components that have direct or indirect product contact)	
Cytotoxicity	No reactivity. Non-cytotoxic.
Intracutaneous Reactivity	No reactivity. Non-irritant.
Systemic Toxicity	No reactivity. Non-toxic.
Toxicology Studies (DERMAGRAFT)	
Genotoxicity assays: Ames Mutagenicity Mouse Lymphoma Mutagenesis	 Pass. Non-mutagenic. Pass. Non-mutagenic.
Tumorigenicity	Evaluation of tumor formation of end of production stage fibroblasts in nude athymic mice revealed the cells to be non-tumorigenic.
Stability/Shipping Studies	
Stability of the device when stored at –70.	Stability testing via assays to evaluate the product following real-time aging. The device when stored at -70°C meets requirements for sterility and product specifications for periods up to seven months.
Validation study to verify that the manufacturing process consistently produces DERMAGRAFT.	Three consecutive qualified validation lots passed initial release criteria and established a minimum of three months acceptable stability. Long term testing results establish stability of the product for up to six months when stored at -75°C ± 10°C was obtained.
Safe shipping of DERMAGRAFT	Thermal, impact and airfreight shipping tests were conducted to assure storage temperature requirements are maintained and all product release criteria are met with respect to sterility, viability, collagen and DNA. DERMAGRAFT can be safely shipped to domestic and international shipping destinations.

Stability/Shipping Studies	
Product Equivalency for Commercial Scale Manufacturing	Demonstrated that product evaluated in pilot and first pivotal clinical studies (4"x6") is equivalent to commercial scale (2"x3") product determined by similarity of critical product characteristics and process trends.

The fibroblast cells, from which DERMAGRAFT is manufactured, are from human infant foreskin tissue. A comprehensive medical history of the mother is taken and maternal serum is screened for the presence of HIV-1, HIV-2, HTLV-I, Hepatitis B core antigen, Hepatitis B surface antigen, Hepatitis C, non-A/non-B Hepatitis (detection via Alanine Aminotransferase (ALT) activity), Cytomegalovirus (CMV), and Syphilis. Additionally, the human foreskin fibroblasts used to form DERMAGRAFT are derived from cell banks which are tested for the presence of HIV-1, HIV-2, HTLV-I, HTLV-II, Hepatitis B, Hepatitis C, Herpes Simplex Virus (HSV) Types 1 and 2, CMV, Epstein Barr Virus (EBV), Adenovirus, Adventitious Viral Contaminants (*In Vitro* and *In Vivo* Assays), virus particles by Electron Microscopy (EM), retrovirus by RT-PCR, mycoplasma, bacteria, and fungi.

Karyological analyses of the fibroblast cells used in manufacture of DERMAGRAFT revealed a limited number of chromosomal changes. These same cells did not exhibit a transformed phenotype in *in vitro* nor in *in vivo* tumorigenicity assays. Cell growth characteristics, i.e., morphology, cell doubling time, and substratum adherence, were monitored and judged to be consistent through end of production stage cells. Isoenzyme purity analyses and DNA marker identification evaluations indicated that the cell bank was from a sole donor.

Product manufacture includes the use of bovine calf serum (BCS). The BCS is provided with a certificate of analysis documenting the viruses screened for in the serum and that it is obtained from a Bovine Spongiform Encephalopathy (BSE)-free country. The sponsor, upon receipt of BCS, tests the serum for sterility, endotoxin and mycoplasma. The serum is functionally assessed by a growth promotion test.

To maintain cell viability the product is aseptically manufactured, but not terminally sterilized. DERMAGRAFT is shipped after quarantine pending results of sterility (14 day), endotoxin and mycoplasma testing.

IX. SUMMARY OF THE RESULTS OF THE CLINICAL INVESTIGATION

Advanced Tissue Sciences, Inc. has conducted 5 trials in the investigation of DERMAGRAFT as a human replacement dermis in the treatment of diabetic foot ulcers. Table 3 summarizes the clinical experience of DERMAGRAFT.

Table 3
Summary of Clinical Trial Experience with DERMAGRAFT

Protocol Number	Description	Length of Follow-up	Number of Patients Enrolled	Number of Devices Applied
DG-04-02-0293	Feasibility	12 Weeks	50	177
DG-04-04-0694	Pivotal I	32 Weeks	281	925
DG-04-04-0694	Supplementary	32 Weeks	50	323
DG-04-07-0798	Pivotal II	12 Weeks	314	927
DG-04-08-0998	Treatment IDE	20 Weeks	49	369
TOTAL			744	2721

FEASIBILITY STUDY

From March 1993 to March 1994, a feasibility study (DG-04-02-0293) was conducted to determine the most appropriate application regimen of DERMAGRAFT for the treatment of diabetic foot ulcers. A total of 50 patients were enrolled at five sites in the 12 week trial. Patients were masked and randomized to one of four treatment arms: one piece of DERMAGRAFT applied to the wound weekly for a total of eight applications (A), one piece of DERMAGRAFT applied to the wound every two weeks for a total of four applications (B), two pieces of DERMAGRAFT applied to the wound every two weeks for a total of eight applications (C), or control treatment consisting of conventional therapy (D). In group A, 50% of the patients achieved complete wound closure compared to 8% for the Control group. Those patients randomized to treatment groups B and C were also found to heal more often than those in the Control group. Further, there was a dose dependent increase in wound closure.

MULTI-CENTER TRIAL I

From September 1994 to January 1997, a pivotal study (DG-04-04-0694) was conducted to evaluate safety and effectiveness. A total of 281 patients across 20 centers were enrolled in the 32 week trial. Patients were masked and randomized to either one piece of DERMAGRAFT applied to the wound weekly for a total of eight applications or to control treatment consisting of conventional therapy. This study identified a specific range of metabolic activity, as measured by MTT reductase activity which determined the final metabolic release criterion that was utilized in the subsequent supplemental and pivotal studies.

In the Intent-to-Treat analysis, 42 of the 139 DERMAGRAFT patients (30%) and 40 of the 142 control patients (28%) healed at 12 weeks. Before 12 weeks of follow-up were obtained, 30 DERMAGRAFT and 16 control patients were discontinued.

All the patients who completed the 12-week efficacy evaluation period of the study or reached complete healing prior to Week 12 were considered evaluable for the efficacy analysis. Patients who were discontinued from the study prior to Week 5 of the efficacy period were deleted from the analysis. 42 of the 109 evaluable patients from the DERMAGRAFT group (39%) and 40 of the 126 evaluable patients from the control group (32%) reached complete wound closure by 12 weeks.

For adverse events by week 12, there were 8 DERMAGRAFT patients and four control patients with study wound osteomyelitis, 8 DERMAGRAFT patients and 9 control patients with study wound cellulitis, 30 DERMAGRAFT patients and 34 control patients with study wound infection, 11 DERMAGRAFT patients and 6 control patients had study wound-related surgery.

SUPPLEMENTARY STUDY

In January 1997, a subsequent clinical trial was conducted to study patients treated with DERMAGRAFT with a specific range of metabolic activity, as measured by MTT reductase activity (protocol number DG-04-04-0694). Fifty patients were enrolled across 10 centers in the 32 week trial. Patients were not masked and all were treated with one piece of DERMAGRAFT applied to the wound weekly for a total of eight applications.

In an intent-to-treat analysis, 20 of 50 DERMAGRAFT patients (40%) achieved wound closure at 12 weeks. Complete wound closure by week 12 data was observed in 51.3% of the evaluable patients. These effectiveness analyses were based on patients with ulcers of any duration.

TREATMENT IDE

The primary objectives of this study was to (1) gain experience on the use of DERMAGRAFT; and (2) to expand health care provider access to DERMAGRAFT while the pivotal trial for this device was conducted. This study was an open label, multicenter study to evaluate the use of DERMAGRAFT in patients with foot ulcers deemed to be of diabetic etiology on the basis of a medical history and a physical examination. Patient's wounds in this study were treated with up to 8 applications of DERMAGRAFT, plus conventional treatment, (debridement, infection control, a covering with Dermanet followed by either a saline moistened gauze or foam dressing). It was up to the Investigator's discretion as to which type of dressing (either foam or saline-moistened gauze) he/she chose for the patient. However, the dressing type remained the same for the patient throughout the course of the study. Patients were followed for 20 weeks.

As of April 4, 2001, a total of 36 centers received IRB approval. Of these, 22 centers actively recruited patients for enrollment, and 49 subjects had been enrolled into this trial. To date, one death, unrelated to the use of DERMAGRAFT, was reported. There were no reported unanticipated adverse device effects. A total of 383 devices have been shipped, a total of 369 pieces implanted, and 14 pieces of product returned to Advanced Tissue Sciences, Inc.

PIVOTAL STUDY

STUDY DESIGN

Patients were randomized to treatment with either DERMAGRAFT plus conventional therapy or conventional therapy alone (debridement, saline-moistened gauze, and pressure-reducing footwear). The criteria for inclusion in the pivotal study were:

- Patient is 18 years old or older.
- Patient has a current diagnosis of Type I or Type II diabetes mellitus.
- Patient's ulcer has been present for a minimum of two weeks under the current Investigator's care.
- Patient's foot ulcer is on the plantar surface of the forefoot (defined as distal to the Tarsal-Metatarsal joint and would include toes) or heel and is greater than or equal to 1.0 cm^2 in size by the ulcer area grid method at Day 0 (the day of randomization). A wound that was greater than 1.0 cm^2 at Screening but has healed to less than 1.0cm^2 at Day 0 is not eligible for this study.
- Patient's ulcer extends through the dermis and into subcutaneous tissue (granulation tissue may be present) but without exposure of muscle, tendon, bone, or joint capsule.
- Patient's wound (after debridement) is free of necrotic debris, exhibits no sign of clinical infection, and appears to be comprised of healthy vascularized tissue.
- Patient's Ankle-Arm Index by Doppler is $\geq$ 0.7.
- The patient has adequate circulation to the foot as evidenced by a palpable pulse on the study foot (either dorsalis pedis or posterior tibial artery).
- If patient is capable of bearing children, she is using a medically accepted means of birth control, and she tests negative on a serum pregnancy test.
- Patient and caregiver are willing to participate in the clinical study and can comply with the follow-up regimen.
- Patient or his/her legal representative has read and signed the Institutional Review Board (IRB) approved Informed Consent form before treatment.
- Patient's study ulcer has been present (open) for > 6 weeks at the time of the Screening visit. (note: This inclusion criteria was approved in February 2000 by the FDA. Enrollment for this study ended before this change could be implemented.)

The criteria for exclusion were:

- Patient has clinical evidence of gangrene on any part of the affected foot.
- Patient's ulcer is over a Charcot deformity of the mid-foot (“Rocker-Bottom Foot") or over the tarsal bones-talus, distal calcaneous, navicular, and cuboid.
- The patient's ulcer is due to a nondiabetic etiology.
- Patient's ulcer has tunnels or sinus tracts that cannot be completely debrided.
- Patient's ulcer has a total surface area that is greater than 20 cm^2.

- Patient's ulcer has decreased in size by 50% or more during the screening period.
- Patient's ulcer has increased in size by 50% or more during the screening period.
- Patient has one or more medical condition(s) that in the opinion of the Investigator would make the patient an inappropriate candidate for this wound healing study.
- Patient has or has had a malignant disease (other than facial basal carcinoma) not in remission for five years or more.
- Patient has severe malnutrition as evidenced by albumin <2.0.
- Patient has known alcohol or drug abuse.
- Patient's random blood sugar reading is greater than 450 mg/dL .
- Patient's urine ketones are noted to be "Small, Moderate, or Large".
- Patient has a nonstudy ulcer on the study foot that is located within 7.0 cm from the study ulcer at Day 0.
- Patient is receiving oral or parenteral corticosteroids, immunosuppressive or cytoxic agents, coumadin, heparin, or is anticipated to require such agents during the course of the study.
- Patient has a history of bleeding disorder.
- Patient has Acquired Immunodeficiency Syndrome (AIDS) or is know to be infected with Human Immunodeficiency Virus (HIV).
- Patient has participated in another study and has been treated with an investigational product within the previous 30 days.
- Patient has any elective osseous procedures to the study foot within 30 days prior to the Screening visit.
- Patient has previously been treated with DERMAGRAFT.
- Patient's ulcer is accompanied by cellulitis, osteomyelitis, or other clinical evidence of infection.
- Patient has any condition(s) which seriously compromises the patient's ability to complete this study.

TREATMENT PROTOCOL

Patients in the DERMAGRAFT group received up to 8 applications of DERMAGRAFT over the course of the 12 week study. All patients received pressure-reducing footwear and were encouraged to stay off their study foot as much as possible. Total off-weighting (e.g., use of crutches and wheelchairs) was not required by the study protocol.

STUDY ENDPOINTS

The primary effectiveness endpoint of this study was complete wound closure by Week 12. Wound closure was defined as full epithelialization without drainage.

The secondary effectiveness endpoints were:

- Time to complete wound closure
- Percent of wound closure by Week 12.

INTERIM ANALYSIS

A planned interim analysis was performed during the study that showed a relationship between ulcer duration at the time of screening and incidence of ulcer healing with DERMAGRAFT. Consequently, a modified (after the interim analysis) statistical plan specified that (1) the effectiveness analyses would be based only on the patients with ulcers greater than 6 weeks in duration at the time of the screening visit and (2) the primary endpoint would be analyzed using Bayesian statistical methods. Bayesian methods provide for information obtained during the initial part of a trial to be utilized prospectively in the latter part of the trial to enable overall estimation of measures of effectiveness. The effectiveness data are therefore based on the 245 patients with ulcers of greater than 6 weeks duration. The safety analyses were performed on all 314 patients who were randomized into the study.

PATIENT ACCOUNTING

For the population of patients with ulcers of any duration, 17% (27/163) of DERMAGRAFT patients left the study prior to completing it. In the control group, 21% (32/151) of patients did not remain in the study. For the population of patients with ulcers greater than six weeks, 16% (21/130) of DERMAGRAFT patients and 22% (25/115) of control patients discontinued prior to completing the study, respectively.

Summaries of patient characteristics are provided in Table 5.

Table 5
Summary of Pivotal Study Patient Characteristics†

Parameter	Treatment Group	N††	Median	Range
Age (years)	DERMAGRAFT	130	55.5	27.0 - 83.0
	Control	115	54.0	31.0 - 79.0
Ulcer Duration (weeks)	DERMAGRAFT	130	25.0	7.0 – 208.0
	Control	115	28.0	7.0 – 999.0
Ulcer Area (cm^2)	DERMAGRAFT	130	1.6	0.8 – 16.7
	Control	115	1.4	0.5 – 18.0
Hemoglobin A1c (%)	DERMAGRAFT	129	8.5	4.6 – 14.0
	Control	114	8.5	4.7 – 16.4
Albumin (g/dL)	DERMAGRAFT	129	4.0	2.9 – 5.1
	Control	115	4.0	2.9 – 5.0
Ankle-Arm Index	DERMAGRAFT	128	1.0	0.6 – 1.7
	Control	114	1.1	0.6 – 1.7
Body Mass Index (kg/m^2)	DERMAGRAFT	130	30.8	18.6 – 54.4
	Control	115	31.5	20.9 – 61.0

† All data obtained at Screening except for Ulcer Area that was obtained at the Day 0 Randomization Visit.
†† Note: Due to missing observations/data, the "N" will vary.

ANALYSIS AND RESULTS

Effectiveness Results

Complete Wound Closure by Week 12

Complete wound closure by Week 12 was determined by the Investigator as a wound that was completely reepithelialized and without drainage. Closed wounds were seen for two consecutive weeks. If the wound had reopened, then it was not considered healed. The Bayesian analysis concluded that the probability that DERMAGRAFT plus conventional therapy increased the chance of achieving wound closure in patients with ulcers greater than 6 weeks in duration over and above that of conventional therapy alone was 98.4%. Furthermore, there is a 95% probability that the chance of achieving closure in patients with ulcers greater than 6 weeks duration ranges from 22% to 38% in the DERMAGRAFT group and 12% to 26% in the Control group.

Percent of Wound Closure by Week 12

Secondary endpoints in the study (percent of wound closure by week 12 and time to reach complete wound closure) were analyzed using frequentist statistical methods.

By Week 12 the median percent wound closure for the DERMAGRAFT group was 91% compared to 78% for the Control group ($p = 0.044$; 2-sided Kruskal-Wallis test).

Time to complete wound closure

The study also showed that ulcers treated with DERMAGRAFT closed significantly faster than ulcers treated with conventional therapy ($p = 0.040$; 2-sided log rank test). Patients treated with

DERMAGRAFT were 1.7 times more likely to close than Control patients at any given time during the study (p = 0.044; 2-sided Cox's proportional hazards model).

Patients reported being ambulatory an average of 8 hours per day.

Patient Demographics and Wound Closure

Summaries of patient characteristics and wound closure are provided in Table 6.

Table 6
Summary of Complete Wound Closure Results by Patient Category for Patients with Wounds of Greater than 6 Weeks Duration[1]

Category	Number and Percent of Wound Closure by 12 Weeks	
	DERMAGRAFT n/N (%)[2]	Control n/N (%)[2]
Age (years) [3]		
=55	17/65 (26.2)	14/63 (22.2)
>55	22/65 (33.8)	7/52 (13.5)
Albumin (g/dL) [3]		
=4.0	24/70 (34.3)	12/67 (17.9)
>4.0	14/59 (23.7)	9/48 (18.8)
Alcohol Use		
Yes	6/37 (16.2)	5/28 (17.9)
No	33/93 (35.5)	16/87 (18.4)
Ankle-Arm Index [3]		
=1.1	20/70 (28.6)	12/54 (22.2)
>1.1	18/58 (31.0)	9/60 (15.0)
Body Mass Index (kg/m^2) [3]		
=31.1	21/68 (30.9)	14/55 (25.4)
>31.1	18/62 (29.0)	7/60 (11.7)
Diabetes Type		
Type I	8/32 (25.0)	5/27 (18.5)
Type II	31/98 (31.6)	16/88 (18.2)
Gender		
Male	22/90 (24.4)	15/91 (16.5)
Female	17/40 (42.5)	6/24 (25.0)
Hemoglobin A1c (%) [3]		
=8.5	19/65 (29.2)	13/58 (22.4)
>8.5	20/64 (31.2)	8/56 (14.3)
Mean Hours Non-Weight Bearing [3]		
=15.7	15/54 (27.8)	13/58 (22.4)
>15.7	21/65 (32.3)	7/47 (14.9)
Number of Ulcers on Study Foot		
1	37/126 (29.4)	20/108 (18.5)
>1	2/4 (50.0)	1/7 (14.3)
Race		
Caucasian	27/90 (30.0)	16/87 (18.4)
Non-Caucasian	12/40 (30.0)	5/28 (17.9)
Smoker		
Yes	8/27 (29.6)	4/17 (23.5)
No	31/103 (30.1)	17/98 (17.3)
Ulcer Area (cm^2) [3]		

=1.5	24/60 (40.0)	15/63 (23.8)
>1.5	15/70 (21.4)	6/52 (11.5)
Ulcer Location		
Forefoot or Toe	33/112 (29.5)	20/102 (19.6)
Heel	6/18 (33.3)	1/13 (7.7)

[1] Data observed at Screening except for Ulcer Area (obtained at the day 0 randomization visit) and Mean Hours Non-weight Bearing (compiled from patient diary information received from Study Weeks 1 through Termination; patients were included if they turned in at least one diary from any post randomization visit).

[2] Note: For individual categories the N will vary based on available patient information.

[3] Cut-off values for each category are based on the overall median value.

The healing results presented in Table 6 above are presented for general information purposes only. Outcome data based on an analysis of one demographic parameter in isolation may not be predictive of wound closure, as multiple factors influence ulcer healing.

Table 7 represents the wound closure results according to the duration of the ulcer.

Table 7
Summary of Complete Wound Closure Results by Ulcer Duration

Ulcer Duration	Number and Percent of Wound Closure by 12 Weeks	
	DERMAGRAFT n/N (%)	**Control n/N (%)**
<6 weeks [1]	13/33 (39.0)	15/36 (42.0)
6 –26 weeks	19/68 (27.9)	11/55 (20.0)
>26 weeks	20/62 (32.3)	10/60 (16.7)

[1] These 69 patients with ulcers less than 6 weeks in duration were not included in the primary effectiveness analysis.

Ulcer Recurrence

In the previous multi-center controlled trial 139 patients were treated with DERMAGRAFT and 142 patients were treated with control. All patients were followed to week 32. Ulcer recurrence (defined as ulcers that healed by week 12 and reopened on or before week 32) was 26% (11/42) for patients in the DERMAGRAFT group and 22% (9/41) for patients in the Control group. Among this group of patients that experienced recurrence, the median time from healing to recurrence was 10 weeks for the DERMAGRAFT group, and 7 weeks for the Control group. These results are reflective of the entire study population, regardless of ulcer duration, and include patients who received DERMAGRAFT that did not meet the final metabolic release criterion.

After this study was completed, the metabolic release criterion for DERMAGRAFT and the intended patient population were modified. Therefore, a retrospective analysis was also performed on a subset of patients with ulcer duration of greater than 6 weeks who received DERMAGRAFT that met the final metabolic release criterion versus Control patients with ulcer duration of greater than 6 weeks. Ulcer recurrence was 18.8% (3/16) for patients in the DERMAGRAFT group and 20.7% (6/29) for patients in the Control group.

Safety Results

Adverse events are displayed in Section VI for the five studies with the product. The adverse event experience for DERMAGRAFT patients and control patients were comparable for most adverse events.

Immunology and Persistence Studies

The potential for DERMAGRAFT to elicit an immune response was evaluated by examining the baseline and terminal sera of patients enrolled in a clinical trial for DERMAGRAFT using Western Blot technique. A comparison of pre- and post-immune sera did not indicate an immunologic response to DERMAGRAFT in patients treated with up to 8 pieces of DERMAGRAFT. In investigating the persistence of the product in the wound bed, testing using Y-chromosome [male donor] marker SRY, amplified by a nested PCR technique revealed the presence of DERMAGRAFT cells from biopsies of treated venous ulcers up to 6 months after treatment from a single piece of DERMAGRAFT. Six of 10 patients evaluated at 2 months demonstrated DNA from DERMAGRAFT cells. Three of 10 patients evaluated at 6 months demonstrated DNA from DERMAGRAFT cells. In addition, biopsies of these wounds were evaluated for histologic evidence of an immunologic response to the product. This assessment found no histologic changes suggestive of an immune response to DERMAGRAFT.

X. CONCLUSIONS DRAWN FROM THE STUDIES

These studies provide reasonable assurance of the safety and effectiveness of DERMAGRAFT for the treatment of full-thickness diabetic foot ulcers greater than six weeks duration.

The preclinical studies demonstrate that the device is composed of biocompatible components.

The probability that DERMAGRAFT plus conventional therapy provided a treatment benefit to patients with ulcers greater than six weeks over and above that of conventional therapy alone is 98.4%. Furthermore, there is a 95% probability that the percent of patients achieving wound closure with DERMAGRAFT ranged from 22% to 38% and that the percent of patients achieving wound closure with Control ranged from 12% to 26%.

Serious Adverse Events- No serious adverse events were attributed to DERMAGRAFT. Of the 314 patients enrolled, 10.4% (17/163) of the DERMAGRAFT patients developed an infection while 17.9% (27/151) of the Control patients developed ulcer infection. Overall, 31/163 (19%) of the DERMAGRAFT group developed infection, cellulitis, or osteomyelitis. In the Control Group, 49/151 (32.5%) patients developed the same adverse events. Thus, there was a lower rate of infection, cellulitis, and osteomyelitis in the DREMAGRAFT treated group.

Immune Response- Analysis of DERMAGRAFT cells showed that there was no antigens attributable to DERMAGRAFT cells.

XI. PANEL RECOMMENDATIONS

The application was not reviewed by the General and Plastic Surgery Devices Panel.

CDRH Decision
Inspections of the sponsor's manufacturing facilities and sterilization sites were completed on January 11, 2001 and were found to be in compliance with the device Good Manufacturing Practice regulations.

The FDA worked with the sponsor to finalize product labeling.

FDA issued an approval order on September 28, 2001

Approval Specifications
Directions for Use: See product labeling.

Hazard to Health from Use of the Device: See Indications, Contraindications, Warnings, and Precautions, Adverse Reactions in the labeling.

Postapproval Requirement and Restrictions: See the approval order.