Confirmatory Data from EU Study Supports Apligraf for the Treatment of Neuropathic Diabetic Foot Ulcers

John S. Steinberg, DPM*
Michael Edmonds, MD, FRCP†
David P. Hurley, Jr., MD‡
William N. King, MS§

Introduction: A study of 72 subjects conducted in the European Union and Australia assessed the safety and efficacy of Apligraf (Organogenesis, Inc, Canton, Massachusetts), a bilayered cell therapy composed of living keratinocytes and living fibroblasts in the treatment of non-infected, diabetic foot ulcers (DFU). The design and patient population of this study were similar to a 208-subject United States study (Veves et al., 2001), which led to FDA approval of Apligraf for the treatment of DFU. EU patient outcomes were compared and contrasted to established US-based patient outcome parameters.

Methods: Subjects with a non-infected neuropathic diabetic foot ulcer present for at least two weeks were enrolled in these prospective, multicenter, randomized, controlled, open-label studies that compared Apligraf used in conjunction with standard therapy (sharp debridement, standard wound care, and off-loading) against standard therapy alone.

Results: The design, conduct, and patient populations of the EU and US studies were comparable. Pooling of data was able to be performed because of the similarity and consistency of the two studies. Efficacy and safety results remained consistent across studies independent of mean ulcer duration that was significantly longer in the EU study (21 months, compared to 10 months in the US). Reported adverse events through 12 weeks were comparable across treatment groups in the two studies. Multiple efficacy measures consistently demonstrated superiority of Apligraf treatment over control treated groups in both studies. Combining the data from both studies, 55.2% (80/145) of Apligraf subjects had complete would closure by 12 weeks, compared to 34.3% (46/134) of Control subjects ($P = 0.0005$; Fisher's exact test), and Apligraf subjects had a significantly shorter time to complete wound closure ($P = 0.0004$; log-rank test).

Conclusions: Both the EU and US studies exhibited superior efficacy and comparable safety for subjects treated with Apligraf compared to control treated subjects. The similar outcomes of the two studies provide robust, consistent evidence of the benefit of Apligraf in treating geographically disparate DFU patient populations.

Introduction

Diabetic foot ulcers (DFU) are a major clinical problem and such ulcers may not respond to standard therapeutic procedures. A person-level meta analysis showed that 67% of patients with DFU will fail to heal (Kantor, 2000). The data presented in this poster compared the efficacy and safety of Apligraf (Organogenesis, Inc, Canton, MA) which is composed of living human keratinocytes and fibroblasts, in the treatment of non-infected, DFU as reported in two studies, one conducted in the United States (US) and one conducted in the European Union and Australia (EU).

Both studies were multicenter, randomized controlled trials using intent to treat (ITT) analysis of data. This poster contains results that was designed to test whether data generated by US-based DFU patients, whose outcomes Organogenesis, Inc used for agency approval of Apligraf, could be independently reproduced in a complimentary EU and Australian DFU bridging study.

The EU study compared the efficacy and safety of Apligraf in combination with international standard therapy guidelines (Apelqvist et al., 1999) versus stan-

*Georgetown University Hospital
†Kings College Hospital
‡Organogenesis
§Western Statistical Consulting

dard therapy alone in the treatment of neuropathic diabetic foot ulcers. The US study compared Apligraf in combination with standard of care at the time (ADA Foot Care Guideline, 1996) with standard of care therapy alone. Efficacy, in both studies, was assessed by time to complete wound healing as well as the incidence of complete wound closure by 12-weeks.

Methods

To determine whether the results of the EU and US studies were comparable, the studies were compared over five major areas which were chosen a priori.

Study design and procedures were compared qualitatively by looking specifically at any differences in inclusion or exclusion criteria and differences in study procedures such as screening, treatment schedule, modalities and follow-up.

The baseline populations in the two studies were compared statistically across multiple characteristics such as age, sex, race, wound size and wound duration and HgbA1c. Appropriate statistical tests were chosen based on the nature of the variable (e.g. continuous, categorical, etc.). The variables chosen were those considered clinically relevant at the time of the design of the two studies and were collected as per study protocols.

Efficacy in the studies was compared on three key measures: 1) time to complete healing as a comparison of the Kaplan-Meier survival functions using the log rank test, 2) incidence of healing at 12 weeks using Fisher's exact test, 3) time to complete healing with comparison of Cox proportional hazards estimate of treatment effect, after adjusting for pertinent covariates.

Recurrence was compared using a two sided Fisher's exact test.

Finally, safety was compared in the two studies on the occurrence of three specific events: infection, osteomyelitis, and amputation. Each of these was tested using a two sided Fisher's exact test. After noting no difference between study groups on these safety variables, data were pooled for analysis by a Cochran-Mantel-Haenszel model using the Breslow-Day test, where possible, to determine if there was evidence of a treatment by study group location interaction.

Results

EU subject recruitment began in 2000 with last follow-up in 2002. Sponsor directed premature closure to enrollment for non-safety related reasons. Initial study design based on US study power analysis was for 240 subject enrollment at 80% power for 2-sided X2 test with 5% type I error.

EU study patients: 106 subjects; 82 randomized; 72 treated; 33 Apligraf and 39 Control.

EU study specific outcomes:

Apligraf Treatments: Average number of applications was 1.8 during the 12-weeks

Single Treatment:	13/33
Double Treatment:	15/33
Triple Treatment:	5/33

Debridements: Mean was 7.2 and 8.6 in the Apligraf and Controls, respectively.

Apligraf: More subjects did not require debridement following initial or 4-week reapplication ($P <$ 0.001 and 0.0273, respectively).

Adverse Events: 10 SAE's were reported during the treatment period, with 1 death

Apligraf: 4 subjects, 1 death due to MI, no allergies reported

Control: 5 subjects

Baseline characteristics were highly similar between treated populations (i.e., age, sex, BMI, wound location, number of ulcers at site, wound size and HbA_1c). There were, however, three statistically significant exceptions:

Race: US study had a more diverse racial profile than the EU study (P <0.0001)

Ulcer Duration: EU study subjects had a longer period of duration of the treated ulcer compared to the US study by approximately 10 months ($P < 0.0001$)

Incidence of Charcot: A subset of US study subjects had inactive Charcot's deformity, whereas none were treated in the EU study ($P < 0.0001$)

Since the EU study was stopped before full enrollment, results from this study are underpowered for demonstrating statistically significant differences. However, even though the study was halted prematurely, results from the EU study are similar to those of the US study and in particular the EU study showed significant ($P = 0.049$, Fisher's exact test) superiority of Apligraf to achieve complete healing at 12-weeks follow-up in comparison to the control group.

In summary, these data show consistent findings for the comparable safety of Apligraf (as compared to Control) in both the US and EU studies, as well as ulcer recurrence, and is a supportive study that bridges the pivotal US study for the EU population.

EU vs. US Study Comparisons

Combining data from both studies, 55.2% (80/145) of Apligraf subjects had complete healing by 12 weeks, compared to 34.3% (46/134) of Control subjects. A two-sided Fisher's exact test was used to test for a difference between the groups, yielding a P-value of

Table 1. Summary of Standard Therapy Utilized in EU and US Study Procedures

EU Study	US Study	Discussion
Screening Visit		
• Sharp debridement of the ulcer.	• Sharp debridement of the ulcer.	• Same.
Treatment Visit		
• Debridement prior to treatment.	• Debridement prior to treatment.	• Same.
• Apligraf® was applied directly to ulcer, which was covered with saline-moistened non-adherent Mepitel®. Mepitel was secured by hypoallergenic tape, covered with secondary dressings of saline-moistened gauze, dry gauze, and secured with hypoallergenic tape or Kling™.	• Apligraf® was applied directly to ulcer, which was covered with saline-moistened non-adherent Tegapore®, secured with hypo-allergenic tape, then covered with a secondary dressing of a layer of dry gauze, a layer of petrolatum gauze, and Kling™.	• Both studies utilized a non-adherent primary dressing and moistened dressings.
• Control subjects had Mepitel® applied directly to the ulcer and covered with saline-moistened dressing (as described for the Apligraf® treatment group).	• Control subjects ulcer was covered directly with saline-moistened Tegapore secured by hypoallergenic tape, covered with secondary dressings of a layer of dry gauze, petrolatum gauze, & Kling.	• Both studies utilized a non-adherent primary dressing and moistened dressings.
Treatment Phase		
• Subjects were seen by the investigator at weeks 0.5, 1, 2, 3, 4, 4.5, 5, 6, 7, 8, 8.5, 9, 10, 11, and 12.	• Subjects were seen by the investigator at weeks 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, and 12.	• In the US study, FDA concerns about infection and required mid-weekly visits through week 5.
• Complete dressing changes were performed by the investigator at the week 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 visits.	• Complete dressing changes were performed by the investigator at the week 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 4.5 visits.	• Complete dressing changes by investigator were less frequent in EU study but for longer duration, consistent with study guidelines.
• Investigator secondary dressing changes was performed at the week 0.5 and 1 visits, and at 4.5, and 8.5 visits if wound was open. • Subjects changed secondary dressing once daily until the 12 week visit, except periods between day 0 & 0.5 week visit, the 4 & 4.5 week visit, the 8 & 8.5 week visit.	• For Apligraf® subjects between visits, subjects changed secondary dressing once daily. Ulcers not healed by week 5 were covered with the secondary dressing, and this was changed twice daily until week 12. • For Controls, the subject changed the secondary dressing twice daily from day 0 to the week 12 visit.	• The requirement for secondary dressing changes by the investigator or subject was also consistent with the international practical guidelines.
• Subjects could use crutches, wheelchair and wear customized Aircast Walkers through complete wound closure or week 12. Customized rocker bottom shoe & total contact insole was made/ given at baseline. Shoe worn on non-target foot & then both feet once healed.	• Subjects were asked to use crutches or a wheelchair for the first six weeks of the study. Customized tri-density sandals were worn through the week 12 visit, or for a minimum of 4 weeks after complete wound closure.	• Duration for use of crutches or wheelchair was longer in the EU study, but off-loading could be stopped at complete wound closure, whereas the US study required a minimum of 4 weeks after complete wound closure.
Follow-Up Phase		
• From week 12 to the week 24 visit.	• From week 12 to 6 month visit.	• Same.

0.0005. This *P*-value indicates very strong statistical evidence for the superior performance of Apligraf.

To assess the effect of the treatment while accounting for the study location (i.e., EU or US), a Cochran-Mantel-Haenszel model was employed, controlling for study location as a covariate to predict the effect of treatment group on the time to complete wound closure. After study location was factored into the model, the maximum likelihood *P*-value for treatment group was 0.0005, once again showing very strong statistical evidence of the superiority of Apligraf.

The Breslow-Day test for homogeneity of odds ratios was used in conjunction with the Cochran-Mantel-Haenszel test to determine if there was supportive evidence of a treatment-by-study-location interaction. This test did not find a statistically significant treatment-by-study-location interaction ($P = 0.5721$), indicating a lack of statistical evidence supporting the hypothesis that the treatment effects seen in the US and EU studies are different. Thus, the supportive EU bridging study demonstrates the superior results of effectiveness in the Apligraf treatment group, confirming the results from the pivotal US study in an EU population.

Conclusions

Studies conducted in the US and EU show remarkable similarity in: 1) Efficacy of Apligraf in the treat-

Table 2. Patient Demographics: Current Study and US Study Populations

	Apligraf®		*Control*	
	EU Study	**US Study**	**EU Study**	**US Study**
N	33	112	39	96
Age (years)				
Mean (Std Dev)	56.8 (11.6)	58.3 (10.3)	61.2 (9.7)	56.2 (9.6)
Sex				
Male	12%	21%	15%	23%
Female	88%	79%	84%	77%
Race				
Caucasian	100%	69%	100%	70%
Black	-	18%	-	15%
Hispanic	-	13%	-	14%
Other	-	1%	-	2%
BMI (kg/m^2)				
Mean (Std Dev)	30.9 (4.8)	31.1 (6.6)	31.2 (7.3)	33.4 (7.8)
Wound Duration (months)				
Mean (Std Dev)	23.5 (27.0)	11.5 (13.3)	19.8 (21.3)	11.1 (12.5)
Wound Location				
Toes	15%	20%	26%	14%
Metatarsals	61%	52%	56%	51%
Mid-Foot	24%	27%	18%	35%
Other	-	2%	-	-
Numbers of Ulcers at Site				
One	85%	89%	89%	94%
More than One	15%	11%	11%	6%
Wound Size (cm^2)				
Mean (Std Dev)	3.04 (2.03)	2.95 (3.09)	2.95 (2.06)	2.83 (2.45)
HbA_{1c} (%)				
Mean (Std Dev)	8.4 (1.8)	8.6 (1.5)	8.5 (1.8)	8.6 (1.4)
Charcot's Deformity				
Absent	100%	85%	100%	77%
Inactive	-	15%	-	23%

Note: BMI is missing for two US Apligraf® subjects and two EU Apligraf® subjects.

Table 3. Primary and Secondary Efficacy Outcomes in US and EU Study Populations

Study	**Kaplan-Meier Time to Complete Wound Closure: Log-Rank Test p-value for Treatment (Mean % Closed) ***	**12 Week Incidence of Complete Would Closure: Fisher's Exact Test p-value for Treatment (Mean % Closed) ***	**Cox Model Time to Complete Wound Closure: Likelihood Ratio Test p-value for Treatment**
Pivotal US	0.0026 (55.6%)	0.0082 (56.3%)	0.0023
EU Bridging	0.0591 (54.1%)	0.0490 (51.5%)	0.0694
Combined	0.0004	0.0005	0.0002

Table 4. Ulcer Recurrence Rates through 6 Months in US & EU Study Populations

Study	Ulcer Recurrence: Apligraf®	Ulcer Recurrence: Control	Fisher's exact test p-value
US	39.7% (25/63)	44.4% (16/36)	0.6759
EU	50.0% (8/16)	60.0% (6/10)	0.7015
Combined	41.8% (33/79)	47.8% (22/46)	0.5768

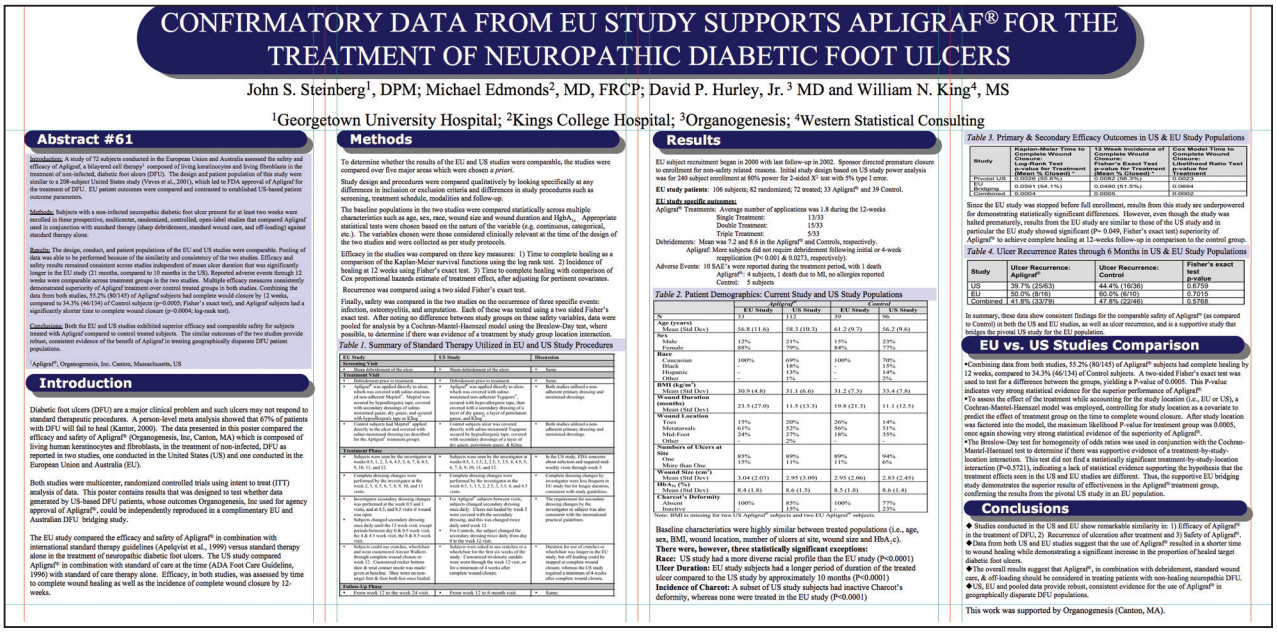
ment of DFU, 2) Recurrence of ulceration after treatment, and 3) Safety of Apligraf.

Data from both US and EU studies suggest that the use of Apligraf resulted in a shorter time to wound healing while demonstrating a significant increase in the proportion of healed target diabetic foot ulcers.

The overall results suggest that Apligraf, in combination with debridement, standard wound care, and off-loading should be considered in treating patients with non-healing neuropathic DFU.

US, EU and pooled data provide robust, consistent evidence for the use of Apligraf in geographically disparate DFU populations.

This work was supported by Organogenesis (Canton, MA).



CONFIRMATORY DATA FROM EU STUDY SUPPORTS APLIGRAF® FOR THE TREATMENT OF NEUROPATHIC DIABETIC FOOT ULCERS

John S. Steinberg[1], DPM; Michael Edmonds[2], MD, FRCP; David P. Hurley, Jr.[3] MD and William N. King[4], MS

[1]Georgetown University Hospital; [2]Kings College Hospital; [3]Organogenesis; [4]Western Statistical Consulting

Abstract #61

Introduction: A study of 72 subjects conducted in the European Union and Australia assessed the safety and efficacy of Apligraf, a bilayered cell therapy[1] composed of living keratinocytes and living fibroblasts in the treatment of non-infected, diabetic foot ulcers (DFU). The design and patient population of this study were similar to a 208-subject United States study (Veves et al., 2001), which led to FDA approval of Apligraf for the treatment of DFU. EU patient outcomes were compared and contrasted to established US-based patient outcome parameters.

Methods: Subjects with a non-infected neuropathic diabetic foot ulcer present for at least two weeks were enrolled in these prospective, multicenter, randomized, controlled, open-label studies that compared Apligraf used in conjunction with standard therapy (sharp debridement, standard wound care, and off-loading) against standard therapy alone.

Results: The design, conduct, and patient populations of the EU and US studies were comparable. Pooling of data was able to be performed because of the similarity and consistency of the two studies. Efficacy and safety results remained consistent across studies independent of mean ulcer duration that was significantly longer in the EU study (21 months, compared to 10 months in the US). Reported adverse events through 12 weeks were comparable across treatment groups in the two studies. Multiple efficacy measures consistently demonstrated superiority of Apligraf treatment over control treated groups in both studies. Combining the data from both studies, 55.2% (80/145) of Apligraf subjects had complete wound closure by 12 weeks, compared to 34.3% (46/134) of Control subjects (p<0.0005; Fisher's exact test), and Apligraf subjects had a significantly shorter time to complete wound closure (p=0.0004; log-rank test).

Conclusions: Both the EU and US studies exhibited superior efficacy and comparable safety for subjects treated with Apligraf compared to control treated subjects. The similar outcomes of the two studies provide robust, consistent evidence of the benefit of Apligraf in treating geographically disparate DFU patient populations.

[1]Apligraf®, Organogenesis, Inc. Canton, Massachusetts, US

Introduction

Diabetic foot ulcers (DFU) are a major clinical problem and such ulcers may not respond to standard therapeutic procedures. A person-level meta analysis showed that 67% of patients with DFU will fail to heal (Kantor, 2000). The data presented in this poster compared the efficacy and safety of Apligraf® (Organogenesis, Inc, Canton, MA) which is composed of living human keratinocytes and fibroblasts, in the treatment of non-infected, DFU as reported in two studies, one conducted in the United States (US) and one conducted in the European Union and Australia (EU).

Both studies were multicenter, randomized controlled trials using intent to treat (ITT) analysis of data. This poster contains results that was designed to test whether data generated by US-based DFU patients, whose outcomes Organogenesis, Inc used for agency approval of Apligraf®, could be independently reproduced in a complimentary EU and Australian DFU bridging study.

The EU study compared the efficacy and safety of Apligraf® in combination with international standard therapy guidelines (Apelqvist et al., 1999) versus standard therapy alone in the treatment of neuropathic diabetic foot ulcers. The US study compared Apligraf® in combination with standard of care at the time (ADA Foot Care Guideline, 1996) with standard of care therapy alone. Efficacy, in both studies, was assessed by time to complete wound healing as well as the incidence of complete wound closure by 12-weeks.

Methods

To determine whether the results of the EU and US studies were comparable, the studies were compared over five major areas which were chosen *a priori*.

Study design and procedures were compared qualitatively by looking specifically at any differences in inclusion or exclusion criteria and differences in study procedures such as screening, treatment schedule, modalities and follow-up.

The baseline populations in the two studies were compared statistically across multiple characteristics such as age, sex, race, wound size and wound duration and $HgbA_{1c}$. Appropriate statistical tests were chosen based on the nature of the variable (e.g. continuous, categorical, etc.). The variables chosen were those considered clinically relevant at the time of the design of the two studies and were collected as per study protocols.

Efficacy in the studies was compared on three key measures: 1) Time to complete healing as a comparison of the Kaplan-Meier survival functions using the log rank test. 2) Incidence of healing at 12 weeks using Fisher's exact test. 3) Time to complete healing with comparison of Cox proportional hazards estimate of treatment effect, after adjusting for pertinent covariates.

Recurrence was compared using a two sided Fisher's exact test.

Finally, safety was compared in the two studies on the occurrence of three specific events: infection, osteomyelitis, and amputation. Each of these was tested using a two sided Fisher's exact test. After noting no difference between study groups on these safety variables, data were pooled for analysis by a Cochran-Mantel-Haenszel model using the Breslow-Day test, where possible, to determine if there was evidence of a treatment by study group location interaction.

Table 1. Summary of Standard Therapy Utilized in EU and US Study Procedures

	EU Study	US Study	Discussion
Screening Visit			
	• Sharp debridement of the ulcer.	• Sharp debridement of the ulcer.	• Same.
Treatment Visit			
	• Debridement prior to treatment.	• Debridement prior to treatment.	• Both
	• Control subjects had Mepitel® applied to the ulcer, which was covered with saline-moistened gauze and Kling.	• Control subjects had Mepitel® applied to the ulcer, which was covered with saline-moistened gauze and Kling.	• Both studies utilized a non-adherent primary dressing and moistened dressings.
	• Control subjects had Mepitel® applied to the ulcer, which was covered with saline-moistened dressing (as described for the Apligraf® treatment group).	• Control subjects ulcer was covered with saline-moistened gauze, covered secure by hypoallergenic tape, covered with secondary dressings of a layer of dry gauze, antiobiotic dress & Kling.	• Both studies utilized a non-adherent primary dressing and moistened dressings.
Treatment Phase			
	• Subjects were seen by the investigator at weeks 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.	• Subjects were seen by the investigator at weeks 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, and 12.	• In the US study, FDA concerns about infection and required and weekly visits through week 5.
	• Complete dressing changes were performed by the investigator at the week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 visits.	• Complete dressing changes were performed by the investigator at the week 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 4.5 visits.	• Complete dressing changes by investigator were less frequent in EU study but for longer duration, consistent with study guidelines.
	• Investigator secondary dressing changes was performed at the week 0.5 and 1 visits, and at 4.5, and 8.5 visits if wound was open.	• Apligraf® subjects between visits without changed secondary dressing once daily. Ulcers not healed by week 5 were covered with the secondary dressing, and this was changed twice weekly until week 12.	• No requirement for the secondary dressing to be done by the investigator or subject was also consistent with the international practical guidelines.
	• Subjects changed secondary dressing daily (Monday through Friday, except period between day 8 & 85 if week visit, the 8 & 8.5 week visit, the 8 & 8.5 week visit).	• For Controls, the subject changed the secondary dressing twice daily from day 1 to week 12.	
	• Subjects could use crutches, wheelchair and wear customized Aircast Walkers through complete wound closure or week 12. Customized rocker bottom shoes, healing sandals and crutches given at baseline. Shoe worn on non-target foot & then both feet once healed.	• Subjects with diabetic foot ulcers were to use a wheelchair for the first six weeks of the study. Customized rocker bottom sandals were worn through the week 12 visit, or as determined at weeks after complete wound closure.	• Duration for use of crutches or wheelchair was longer in the EU study, but off-loading could be stopped at complete wound closure in both studies. EU required a minimum of 4 weeks after complete wound closure.
Follow-Up Phase			
	• From week 12 to the week 24 visit.	• From week 12 to 6 month visit	• Same

Results

EU subject recruitment began in 2000 with last follow-up in 2002. Sponsor directed premature closure to enrollment for non-safety related reasons. Initial study design based on US study power analysis was for 240 subject enrollment at 80% power for 2-sided X^2 test with 5% type I error.

EU study patients: 106 subjects; 82 randomized; 72 treated; 33 Apligraf® and 39 Control.

EU study specific outcomes:

Apligraf® Treatments: Average number of applications was 1.8 during the 12-weeks
Single Treatment: 13/33
Double Treatment: 15/33
Triple Treatment: 5/33

Debridements: Mean was 7.2 and 8.6 in the Apligraf® and Controls, respectively.
Apligraf: More subjects did not require debridement following initial or 4-week reapplication (P< 0.001 & 0.0273, respectively).

Adverse Events: 10 SAE's were reported during the treatment period, with 1 death
Apligraf®: 4 subjects, 1 death due to MI, no allergies reported
Control: 5 subjects

Table 2. Patient Demographics: Current Study and US Study Populations

	Apligraf®		Control	
	EU Study	US Study	EU Study	US Study
N	33	112	39	56
Age (years)				
Mean (Std Dev)	56.8 (11.6)	58.3 (10.3)	61.2 (9.7)	56.2 (9.6)
Sex				
Male	12%	21%	15%	23%
Female	88%	79%	84%	77%
Race				
Caucasian	100%	69%	100%	70%
Black	-	18%	-	14%
Hispanic	-	11%	-	9%
Other	-	3%	-	2%
BMI (kg/m²)				
Mean (Std Dev)	30.9 (4.8)	31.1 (6.6)	31.2 (7.3)	33.4 (7.8)
Wound Duration (months)				
Mean (Std Dev)	23.5 (27.0)	11.5 (13.3)	19.8 (21.3)	11.1 (12.5)
Wound Location				
Toes	15%	20%	26%	14%
Metatarsals	61%	52%	36%	31%
Mid-Foot	24%	27%	18%	35%
Other	-	2%		
Numbers of Ulcers at Site				
One	85%	89%	89%	94%
More than One	15%	11%	11%	6%
Wound Size (cm²)				
Mean (Std Dev)	3.04 (2.03)	2.95 (3.09)	2.95 (2.06)	2.83 (2.45)
HbA1c				
Mean (Std Dev)	8.4 (1.8)	8.6 (1.5)	8.5 (1.8)	8.6 (1.4)
Charcot's Deformity				
Absent	100%	85%	100%	77%
Intactive		15%		23%

Note: BMI is missing for two US Apligraf® subjects and two EU Apligraf® subjects.

Baseline characteristics were highly similar between treated populations (i.e., age, sex, BMI, wound location, number of ulcers at site, wound size and HbA_1c).

There were, however, three statistically significant exceptions:

Race: US study had a more diverse racial profile than the EU study (P<0.0001)

Ulcer Duration: EU study subjects had a longer period of duration of the treated ulcer compared to the US study by approximately 10 months (P<0.0001)

Incidence of Charcot: A subset of US study subjects had inactive Charcot's deformity, whereas none were treated in the EU study (P<0.0001)

Table 3. Primary & Secondary Efficacy Outcomes in US & EU Study Populations

Study	Kaplan-Meier Time to Complete Wound Closure: Log-Rank Test Apligraf® vs. Control (Mean % Closed) *	12 Week Incidence of Complete Wound Closure: Fisher's Exact Test Apligraf® vs. Control (Mean % Closed) *	Cox Model Time to Complete Wound Closure: Likelihood Ratio Test Apligraf® vs. Control
Pivotal US	0.0026 (58.6%)	0.0082 (58.3%)	0.0053
EU Bridging	0.0591 (54.1%)	0.0490 (51.5%)	0.0694
Combined	0.0004	0.0005	0.0002

Since the EU study was stopped before full enrollment, results from this study are underpowered for demonstrating statistically significant differences. However, even though the study was halted prematurely, results from the EU study are similar to those of the US study and in particular the EU study showed significant (P= 0.049, Fisher's exact test) superiority of Apligraf® to achieve complete healing at 12-weeks follow-up in comparison to the control group.

Table 4. Ulcer Recurrence Rates through 6 Months in US & EU Study Populations

Study	Ulcer Recurrence: Apligraf®	Ulcer Recurrence: Control	Fisher's exact test p-value
US	39.7% (25/63)	44.4% (16/36)	0.6759
EU	50.0% (8/16)	60.0% (6/10)	0.7015
Combined	41.8% (33/79)	47.8% (22/46)	0.5768

In summary, these data show consistent findings for the comparable safety of Apligraf® (as compared to Control) in both the US and EU studies, as well as ulcer recurrence, and is a supportive study that bridges the pivotal US study for the EU population.

EU vs. US Studies Comparison

- Combining data from both studies, 55.2% (80/145) of Apligraf® subjects had complete healing by 12 weeks, compared to 34.3% (46/134) of Control subjects. A two-sided Fisher's exact test was used to test for a difference between the groups, yielding a P-value of 0.0005. This P-value indicates very strong statistical evidence for the superior performance of Apligraf®
- To assess the effect of the treatment while accounting for the study location (i.e., EU or US), a Cochran-Mantel-Haenszel model was employed, controlling for study location as a covariate to predict the effect of treatment group on the time to complete wound closure. After study location was factored into the model, the maximum likelihood P-value for treatment group was 0.0005, once again showing very strong statistical evidence of the superiority of Apligraf®.
- The Breslow-Day test for homogeneity of odds ratios was used in conjunction with the Cochran-Mantel-Haenszel test to determine if there was supportive evidence of a treatment-by-study-location interaction. This test did not find a statistically significant treatment-by-study-location interaction (P=0.5721), indicating a lack of statistical evidence supporting the hypothesis that the treatment effects seen in the US and EU studies are different. Thus, the supportive EU bridging study demonstrates the superior results of effectiveness in the Apligraf® treatment group, confirming the results from the pivotal US study in an EU population.

Conclusions

- Studies conducted in the US and EU show remarkable similarity in: 1) Efficacy of Apligraf® in the treatment of DFU, 2) Recurrence of ulceration after treatment and 3) Safety of Apligraf®.
- Data from both US and EU studies suggest that the use of Apligraf® resulted in a shorter time to wound healing while demonstrating a significant increase in the proportion of healed target diabetic foot ulcers.
- The overall results suggest that Apligraf®, in combination with debridement, standard wound care, & off-loading should be considered in treating patients with non-healing neuropathic DFU.
- US, EU and pooled data provide robust, consistent evidence for the use of Apligraf® in geographically disparate DFU populations.

This work was supported by Organogenesis (Canton, MA).

Poster image as presented at the 2009 APMA Annual Scientific Meeting, Toronto.