

Advanced Biological Therapies for Diabetic Foot Ulcers

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Objective: To assess the clinical use of advanced biological therapies in treating diabetic foot ulcers in practice and the effect of these therapies on time to healing.

Design: A retrospective cohort study.

Setting: A validated wound care database from January 1, 2001, through December 31, 2004.

Participants: Two thousand five hundred seventeen patients with diabetic neuropathic foot ulcers.

Intervention: Patients who received advanced biological therapy (ie, Apligraf, Regranex, or Procuren).

Main Outcome Measure: Time to healing after initial use of advanced biological therapy. This was derived using Kaplan-Meier estimates and the Ederer least squares method after adjusting for covariates, which were assessed using generalized estimating equations and Cox proportional hazards regression modeling.

Results: Advanced biological therapy was used, on average, within 28 days from the first wound clinic visit and associated with a median time to healing of 100 days. Regardless of the advanced biological therapy used, wounds with larger wound area, more severe wound grades, longer duration of wound prior to the first visit, and prolonged time to treatment with advanced biological therapies were significantly associated with longer time to healing. Wounds treated with engineered skin as the first advanced biological therapy were 31.2% more likely to heal than wounds first treated with topical recombinant growth factor ($P < .001$), and 40.0% more likely to heal than those first treated with platelet releasate ($P = .01$). Wound size, wound grade, duration of wound, and time to initiation of advanced biological therapy affected the time to healing.

Conclusions: Advanced biological therapies were used, on average, within 1 month, and improved healing of refractory diabetic foot ulcers. Differences on outcomes among advanced biological therapies were noted.

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FOOT ULCERATION IS A MAJOR complication of diabetes, affecting 12% to 15% of patients with diabetes mellitus during their lifetime.^{1,2}

Diabetic foot ulcers (DFUs) impair quality of life and are a cause of significant morbidity, accounting for 20% of all diabetes-related hospital admissions in the United States.¹ Of the almost 82 000 nontraumatic lower limb amputations performed yearly,^{1,3} DFUs are a major risk factor, preceding 85% of amputations in patients with diabetes in the United States.¹ Amputations are associated, with among other things, increased direct cost of care, ranging from \$22 000 to \$60 000.⁴ Improved and faster healing of a DFU has been shown to reduce the incidence of amputation among patients with diabetes.⁵

Current treatment guidelines recommend standard treatment of a DFU to in-

clude off-loading, debridement, and the restoration of skin perfusion.⁵ Recent advances in basic science research and associated techniques have translated into improvement in the care of difficult-to-heal wounds, from the use of autologous growth factors to the development of recombinant growth factors and bioengineered cell-based therapies. Randomized controlled trials have demonstrated that advanced biological therapies in combination with standard care including off-loading and debridement lead to improved healing of DFU compared with standard care alone⁶⁻⁸ and their use is considered routine in treatment algorithms.⁵ In an attempt to study the use of advanced biological therapies in clinical practice, we analyzed a validated wound healing database from Curative Health Services (CHS) that has been found useful in yielding important clinical information.⁸⁻¹¹

Table 1. Descriptive Statistics for Selected Patient and Wound Characteristics at Baseline

Patient Characteristic	No. (%)					P Value
	Analysis Population (n=2517)	Bilayered Living Cell Therapy ^a (n=446 [17.7])	Becaplermin ^a (n=1892 [75.2%])	Platelet Releasate ^a (n=125 [5.0%])	Becaplermin or Platelet Releasate Followed by Bilayered Living Cell Therapy ^a (n=54 [2.2%])	
Patient age ≥65 y	1022 (40.60)	211 (48.06)	732 (38.83)	53 (42.74)	23 (37.10)	.004
Male sex	1501 (59.63)	254 (57.86)	1125 (59.68)	75 (60.48)	40 (64.52)	.75
Hypertension	149 (5.92)	13 (2.96)	129 (6.84)	5 (4.03)	1 (1.61)	.005
Trauma	84 (3.34)	14 (3.19)	66 (3.50)	2 (1.61)	2 (3.23)	.72
Osteomyelitis	62 (2.46)	4 (0.91)	51 (2.71)	5 (4.03)	1 (1.61)	.09

^aBilayered living cell therapy alone indicates Apligraf, Organogenesis Inc, Canton, Massachusetts; becaplermin alone, Regranex, J&J, New Brunswick, New Jersey; platelet releasate alone, Procuren, Cytomedix, Rockville, Maryland; and becaplermin or platelet releasate followed by bilayered living cell therapy, other and Apligraf.

We analyzed clinical utilization patterns and comparative outcomes of advanced biological therapies in a real-world clinical setting, including bilayered living cell therapy (Apligraf; Organogenesis, Canton, Massachusetts), growth factor therapy with recombinant platelet derived growth factor-BB (becaplermin [Regranex]; J&J, New Brunswick, New Jersey) and platelet releasate (Procuren; Cytomedix, Rockville, Maryland), with regard to wound healing. Human fibroblast-derived dermal substitute (Dermagraft; Advanced BioHealing, La Jolla, California) was not commercially available at the onset of the cohort's observation period, and was not included in the analyses. Specifically, we sought to address the following questions: Does choice of and/or time to use of an advanced biological therapy affect time to wound closure? What, if any, demographic and clinical characteristics are associated with selection of an advanced biological therapy? What demographic and clinical characteristics are associated with the time to complete closure?

METHODS

Given the nature of the data, a waiver for the need for institutional review board approval was obtained. The database used for this analysis is composed of longitudinal follow-up of patients treated in CHS facilities throughout the United States since the database's inception in 1988. The database consists of administrative and patient medical records collected from more than 90 wound care clinics and hospitals in 30 states, previously shown to be a reliable and valid tool to study individuals with a DFU.¹² Patients were included in this analysis if they had a primary diagnosis of neuropathic DFU; were seen at a CHS facility during a 4-year period from January 1, 2001, through December 31, 2004; were treated with at least one advanced biological therapy; and had valid visit dates along with patient and wound identifiers. The algorithm used in this study to define neuropathic DFU was previously validated: ulcers occurring on the plantar surface of the foot or heel of an individual with diabetes who had adequate arterial perfusion.¹² In patients presenting with multiple wounds, analysis was for the first wound only; in this database, the first wound is defined as the most severe wound (in terms of wound grade) evaluated during the patient's first wound appointment (ie, time of registration).¹¹ Patients with venous insufficiency and peripheral vascular disease were excluded from the analysis. Patients treated in the CHS system receive standard treatment (as de-

finied by a clinical practice guideline consistent throughout the CHS system) including off-loading and debridement.

Patients were categorized according to which advanced biological therapy they received first. A priori we determined that patients who received multiple advanced biological therapies sequentially (eg, recombinant growth factor followed by bilayered living cell therapy) would be analyzed as a separate group only if the proportion of the total patient number permitted meaningful analysis (ie, ≥ 2% of the total patient population). Patients were observed from the time of their first wound visit until this wound healed or until date of last visit (ie, the patient was no longer seen at the clinic).

The distributions of patient and wound characteristics at baseline are presented as relative frequencies for categorical variables and medians and interquartile ranges (IQR) for continuous variables. Differences among treatment groups for categorical baseline variables for patient or first wound characteristics were tested for statistical significance using the Fisher exact or χ^2 tests, and differences for continuous variables were evaluated using Kruskal-Wallis tests. To identify possible sources of bias caused by selection of initial treatments for patients and wounds with particular characteristics, generalized estimating equations were used to identify factors associated with first treatment with advanced biological therapy.

The primary analysis sought to evaluate differences among advanced biological therapy groups regarding time to healing relative to initiation of advanced biological therapy for the first wound. A healed wound was defined as 100% epithelialization without any dressing or requiring a dressing only for protection.¹²

Kaplan-Meier curves were derived to describe probabilities of healing over time among treatment groups without regard to covariates. To assess the effect of covariates on time to healing as well as the influence of treatment, potential covariates were those (1) considered potentially clinically relevant from prior studies,^{9,10} (2) identified from generalized estimating equations to be associated ($P < .05$) with use of initial advanced biological therapy, and (3) shown in bivariate Cox proportional hazards regression models to suggest some association ($P < .20$) with healing. The covariates included in the initial model are found in **Table 1**. A monitored backward Cox proportional hazards regression procedure was performed iteratively until the final subset of covariates in the model included only those significant at $P < .05$. From variables in this final model, Cox proportional hazards ratios are derived for exposure (treatment) variables as well as covariates. An estimated survival curve for each treatment accounting for significant covariates was derived using the Ederer least squares method.

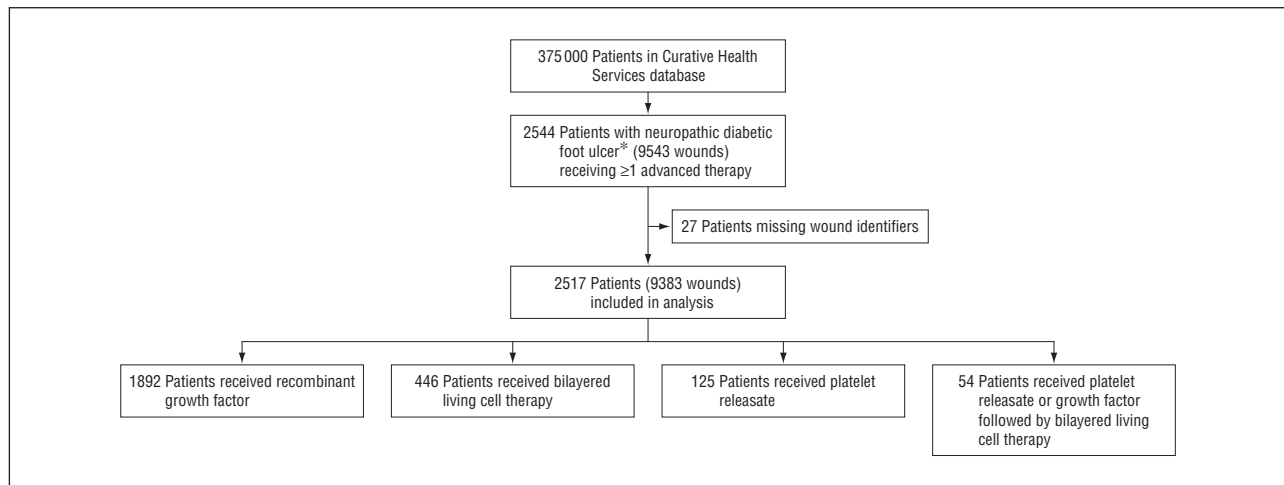


Figure 1. Flowchart depicting the disposition of patient records for inclusion in the analysis. *Patients with neuropathic diabetic foot ulcer were identified using an algorithm previously validated: ulcers occurring on the plantar surface of the foot or heel of an individual with diabetes who had adequate arterial perfusion.¹² Bilayered living cell therapy alone indicates Apligraf, Organogenesis Inc, Canton, Massachusetts; becaplermin alone, Regranex, J&J, New Brunswick, New Jersey; platelet releasate alone, Procuren, CytoMedix, Rockville, Maryland; and becaplermin or platelet releasate followed by bilayered living cell therapy, other and Apligraf.

Sensitivity analyses were performed to determine whether the results of our analysis on the first wounds are consistent for patients who have multiple wounds or who develop subsequent wounds. Using marginal structural Cox proportional hazards regression estimators models for clustered data, the initial marginal structural Cox proportional hazards regression estimator model included the variables in Table 1 with an additional variable indicating rank order of the wound. All tests were 2-tailed and conducted at $\alpha = .05$.

Other secondary analyses using Cox proportional hazards regression models explored the extent to which the timing of advanced biological therapies influenced time to wound healing, regardless of treatment. Specifically, these analyses addressed whether the length of time between registration and start of treatment with an advanced modality was predictive of time to healing.

RESULTS

A total of 2544 patients met the inclusion criterion (primary diagnosis of neuropathic, nonischemic DFU that was treated with at least one advanced biological therapy) (**Figure 1**). As only 27 of 2544 patients (1.1%) were treated with an advanced biological therapy and subsequently treated with growth factor therapy (recombinant growth factor therapy or platelet releasate), these subgroups were considered too small for meaningful analyses. Fifty-four patients who failed initial treatment with growth factor therapy (recombinant growth factor therapy or platelet releasate) and were later treated with bilayered living cell therapy met criteria (sufficiently large) to be categorized as a separate group for analysis. As a result, 2517 patients were included in this analysis and of those patients who constituted the final sample, 1892 patients (75.2%) were treated with recombinant growth factor therapy, 446 patients (17.7%) were treated with bilayered living cell therapy, 125 patients (5.0%) were treated with platelet releasate, and 54 patients (2.1%) were treated with platelet releasate or recombinant growth factor therapy followed by bilayered living cell therapy.

Differences were seen in patient and wound characteristics treated with different advanced modalities first including age of the patient; size, depth, and duration of the wounds; and time from first visit to treatment. Patient and wound demographic information are shown in Table 1 and **Table 2**.

The mean time from first visit to treatment with advanced biological therapy was 28 days, with 25% of the population of patients initiating advanced biological therapy by day 8 and 75% by day 60. Time to use of platelet releasate or recombinant growth factor therapy was more than 2 weeks shorter ($P < .05$) than time to initiation of bilayered living cell therapy (Table 2).

Longer time to healing after first advanced biological therapy was associated across all treatment groups with larger wound area ($P < .001$), more severe wound grade ($P < .001$), longer duration prior to first visit ($P = .003$), and longer time from first visit to use of advanced biological therapy ($P = .001$).

The median time from first use of advanced biological therapy to healing or to last observation was 100 days, with 25% followed up longer than 168 days (maximum follow-up was 1001 days) (**Figure 2**). From Cox proportional hazards regression modeling, wounds treated first with bilayered living cell therapy healed significantly faster ($P < .05$) than when treated with the other 3 advanced biological therapy groups—platelet releasate alone, recombinant growth factor therapy alone, and either platelet releasate or recombinant growth factor therapy followed by bilayered living cell therapy), without regard to confounding factors. When adjusted for potential confounding factors (**Figure 3**), the median time to healing remained faster for bilayered living cell therapy (84 days) compared with 101 days for recombinant growth factor therapy and 108 days for platelet releasate. Hazards ratios and 95% confidence intervals derived from the Cox proportional hazards regression model for time to healing are shown in **Table 3**. Wounds treated with bilayered living cell therapy as the first advanced biological therapy were 31.2% more likely to heal than

Table 2. Baseline Characteristics of the Wound

Wound Characteristic	Median (IQR)					P Value
	Analysis Population (n=2517)	Bilayered Living Cell Therapy ^a (n=446 [17.7%])	Becaplermin ^a (n=1892 [75.2%])	Platelet Releasate ^a (n=125 [5.0%])	Becaplermin or Platelet Releasate Followed by Bilayered Living Cell Therapy ^a (n=54 [2.2%])	
Wound area, mm ²	117 (39-395)	311 (84-925)	141 (49-424)	329 (78-973)	367 (86-1074)	<.001
Wound depth, mm	2 (1-5)	2 (1-5)	2 (1-5)	3 (2-10)	3 (2-6)	<.001
Duration prior to first visit, mo	1 (0.5-3)	2 (0.75-4)	1.5 (0.75-4)	1 (0.5-3)	2 (1-5)	.12
Time from registration to advanced treatment use, d	28 (8-60)	43 (21-96)	23 (7-56)	28 (18-47)	28 (14-47)	<.001
Grade, No. (%) ^b						
0-1	75 (3.00)	12 (2.75)	59 (3.15)	3 (2.44)	1 (1.61)	.25
2	1879 (75.04)	345 (78.95)	1396 (74.45)	89 (72.36)	42 (67.74)	
>2	550 (21.96)	80 (18.31)	420 (22.40)	31 (25.20)	19 (30.65)	

^aBilayered living cell therapy alone indicates Apligraf, Organogenesis Inc, Canton, Massachusetts; becaplermin alone, Regranex, J&J, New Brunswick, New Jersey; platelet releasate alone, Procuren, Cytomedix, Rockville, Maryland; and becaplermin or platelet releasate followed by bilayered living cell therapy, other and Apligraf.

^bThe grades were assessed on a scale of 1 (best) to 6 (worse) based on a proprietary Curative Health Services grading system¹⁰ for outpatient programs and on the Pressure Ulcer Staging System for inpatient care and outreach programs.

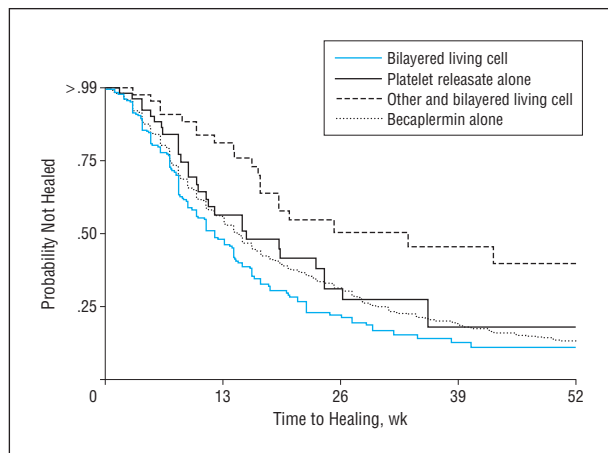


Figure 2. Kaplan-Meier survival curve demonstrates time to heal after treatment with advanced biological therapies for bilayered living cell therapy alone (Apligraf; Organogenesis Inc, Canton, Massachusetts), becaplermin alone (Regranex; J&J, New Brunswick, New Jersey), platelet releasate alone (Procuren; Cytomedix, Rockville, Maryland), and becaplermin or platelet releasate followed by bilayered living cell therapy (other and Apligraf).

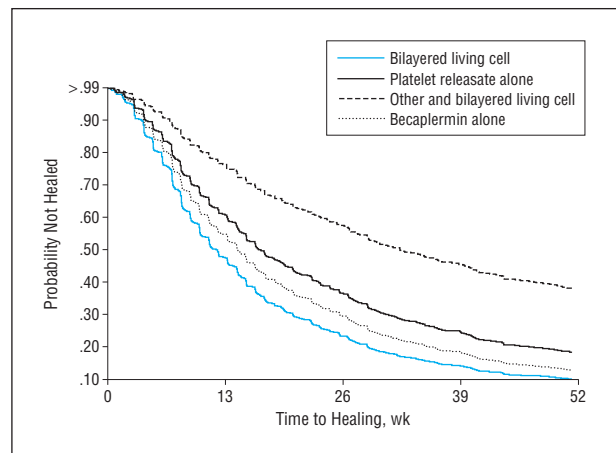


Figure 3. Ederer survival curve demonstrates time to heal after treatment after adjusting for covariates (age, hypertension, wound area, wound depth, and time from registration to advanced treatment use) with advanced biological therapies for bilayered living cell therapy alone (Apligraf; Organogenesis Inc, Canton, Massachusetts), becaplermin alone (Regranex; J&J, New Brunswick, New Jersey), platelet releasate alone (Procuren; Cytomedix, Rockville, Maryland), and becaplermin or platelet releasate followed by bilayered living cell therapy (other and Apligraf).

wounds first treated with recombinant growth factor therapy ($P < .001$), and 40.0% more likely to heal than those first treated with platelet releasate ($P = .01$).

Sensitivity analysis extending analysis to all wounds a patient (ie, multiple wounds and subsequent wounds per patient) demonstrated similar results of the analysis using the first wound with the addition that the wound number (wound assignment number in CHS database that could be multiple or sequential wounds) presented was also significant.

While the CHS data have been previously used and validated, several potential limitations to the use of observational data from a database exist. First, these databases are not typically designed for epidemiological research purposes and, as such, not all variables that emerge in the current literature as potential confounders may have been collected. For example, variables such as glycemic

control, diabetes type, smoking status, variability in off-loading methods of extent of debridement were not adequately controlled for or captured in the database for inclusion into analyses. It is not clear that any bias exists for any of these related to use of a specific advanced wound product. Second, the attention to recording data may not be as rigorous as that for a database designed for research purposes. Third, the limited data-quality review and observed inconsistencies among variables captured across CHS facilities and patients pose additional limitations on validity of results, and, hence, interpretation, although previously analyses have not indicated such a center affect exists. Fourth, progression toward healing impacts the choice of treatment (ie, when advanced biological therapy is only used for chronic, treatment-

resistant wounds), comparisons will be confounded by differences inherent among groups.

COMMENT

We studied the use of advanced biological therapies in clinical practice. We found that among all advanced treatments, larger wound areas, more severe wound grades, longer duration of a wound prior to the first visit, and prolonged time from first visit to treatment with advanced biological therapies were significantly associated with longer time to healing. These factors are similar to those reported in the literature for diabetic foot wounds in general. For example, in patients treated with standard care, larger wound size, longer wound duration, and more severe wound grade have been shown to be significantly negatively associated with the likelihood of wound healing by week 20 of care.¹⁰ The close relationship among ulcer duration, ulcer area, and subsequent outcome emphasize the importance of early expert assessment of newly occurring neuropathic ulcers.¹³

We also demonstrated that differences among the advanced biological therapies existed. Wounds treated with bilayered living cell therapy first were 31.2% more likely to heal, and healed faster than wounds first treated with recombinant growth factor therapy and were 40.0% more likely to heal than those first treated with platelet releasate. It is possible patients and wounds seen at the CHS facilities may be characterized as difficult to heal; CHS facilities specialize in outpatient wound care, and it is possible that presenting patients had previously sought treatment from other health care providers.

Conventional treatment of a DFU, which includes off-loading and debridement, often does not result in complete wound closure for a significant percentage of patients. While some studies have reported better healing rates with imposed off-loading regimens¹⁴ a meta-analysis of patients receiving standard care in randomized controlled trials found that only 31% of DFUs receiving conventional therapy heal within a 20-week period, and only 24% heal within a 12-week period.¹⁵

The Wound Healing Society's published guidelines for the treatment of DFUs recommend a change in the treatment course if a reduction in wound size is not observed after 4 weeks of standard wound therapy.⁵ This is based on studies such as a large, prospective, multicenter trial, performed by Sheehan et al¹⁶ that found patients receiving standard care for a DFU, who did not reduce their wound area more than the 4-week median wound area of 53%, had a decreased likelihood of healing at 12 weeks. Change in the treatment refers to continuing standard care and adding adjuvant treatment. Overall, we found that treatment with advanced biological therapy for nonhealing wounds occurred by day 28, consistent with Wound Healing Society guidelines. However, the median time to use of bilayered living cell therapy was 6 weeks compared with 4 weeks for platelet releasate and 3 weeks for recombinant growth factor therapy. Furthermore, 25% of wounds treated with bilayered living cell therapy were not treated until after 24 weeks. Bilayered living cell therapy was used later than other biological treatment; the reason for this

Table 3. Results of Cox Proportional Hazards Regression Model for Time to Healing in Wounds Treated With Advanced Biological Therapies

Advanced Biological Therapy	Hazard Ratio vs Bilayered Living Cell Therapy (95% CI)	P Value	Time to Healing (Median), d ^a
Platelet releasate only ^b	0.600 (0.402-0.896)	.01	133 (79-168)
Becaplermin only ^b	0.688 (0.572-0.828)	<.001	100 (91-105)
Becaplermin or platelet releasate followed by bilayered living cell therapy ^b	0.337 (0.215-0.529)	<.001	175 (119-488)
Bilayered living cell therapy only ^b	1.000		84 (70-98)
Baseline wound area >117 cm ² , median	0.700 (0.608-0.805)	<.001	NA
Time from first visit to treatment with advanced biological therapy, d	0.997 (0.996-0.998)	<.001	NA
Duration preregistration, d	0.989 (0.983-0.995)	<.001	NA
Grade (0-6) ^c	0.879 (0.811-0.952)	.002	NA

Abbreviations: CI, confidence interval; NA, not applicable.

^aUnadjusted for covariates.

^bBilayered living cell therapy alone indicates Apligraf, Organogenesis Inc, Canton, Massachusetts; becaplermin alone, Regranex, J&J, New Brunswick, New Jersey; platelet releasate alone, Procuren, Cytomedix, Rockville, Maryland; and becaplermin or platelet releasate followed by bilayered living cell therapy, other and Apligraf.

^cThe grades were assessed on a scale of 1 (best) to 6 (worse) based on a proprietary Curative Health Services grading system¹⁰ for outpatient programs and on the Pressure Ulcer Staging System for inpatient care and outreach programs.

is not entirely clear. Whether cost is an issue is not clear; it is possible that cost may be a significant driver for the late use of advanced biological therapies and cause delays in identifying wounds that are nonresponsive to conventional treatment. However, several studies have found that use of advanced biological therapies, in fact, do reduce costs.^{17,18}

This study focused on usage patterns with advanced biological therapies and did not compare these therapies with standard or conventional therapy. All patients included in this analysis had chronic DFUs that had received standard care (debridement and off-loading). Given the retrospective nature of this multicenter study, the treatment regimen prior to advanced biologic therapy varied within the context of patient-centered care. In addition, the history of the wound and therapy received prior to presentation at the CHS facility was not always captured in detail. The results of this study should not be used in isolation when making decisions regarding when to use adjuvant therapy in combination with standard care.

In summary, we found that, in patients enrolled in CHS who received advanced biological therapy, the earlier an advanced biological therapy is initiated, the sooner the wound is likely to heal. Proper treatment is critical for the management of chronic DFUs, and delaying appropriate treatment, when needed, lengthens the time to healing.

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