Pilot Trial of Biovance Collagen-Based Wound Covering for Diabetic Ulcers

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ABSTRACT

BACKGROUND: Diabetes mellitus affects more than 194 million people worldwide, resulting in nearly 40,000 limb amputations per year, secondary to infected ulcerations, peripheral neuropathy, and inadequate vascular status. Diabetic foot ulcers have an underlying metabolic etiology that contributes to a nonhealing ulcer. Biovance (Celgene Cellular Therapeutics, Morris, New Jersey) is a wound covering produced from decellularized, dehydrated human amniotic membrane. The purpose of this study was to determine healing rates for partial- and full-thickness diabetic foot ulcers treated with Biovance. The secondary objective was to determine time to complete wound closure and safety profile.

METHODS: Open-label study of 14 patients with chronic nonhealing diabetic partial- or full-thickness ulcers.

RESULTS: Groups 1 and 2 (55.5% and 33.3%, respectively, comprising 60.1% of total participants) received a benefit from using Biovance wound covering, and there were no adverse reactions to the tissue.

CONCLUSION: Biovance helps decrease healing time for a population of patients with chronic nonhealing diabetic partial- or full-thickness foot ulcers. Randomized, controlled studies may be warranted.

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INTRODUCTION

Diabetes mellitus affects more than 194 million people worldwide, resulting in nearly 40,000 limb amputations per year, secondary to infected ulcerations, peripheral neuropathy, and inadequate vascular status.[1] In 2004, the National Diabetes Surveillance System reported that 14.7 million Americans were diagnosed with diabetes, representing a growth rate of 6.6% as compared with statistics from 1994.[2] Benotmane et al[3] reported that the overall cost expenditure of hospital stay for a total of 163 diabetic foot lesions amounted to $914,534.39. Individualized, this cost amounts to nearly $30,000 within the first 2 years a patient is diagnosed with diabetes and a foot ulceration.[3]

Davis[4] is credited for the use of fetal membranes in skin transplantation. Trelford[5] reported the cases of Stern and Sabella having treated burn victims with amniotic membrane. Their reasoning was that the ectodermal origin of the membrane offered contact with a plethora of fibroblasts and collagen matrices. Biovance (Celgene Cellular Therapeutics, Morris, New Jersey) is a decellularized, dehydrated human amniotic membrane indicated for the management of noninfected partial- and full-thickness wounds. The purpose of this study was to determine healing rates for partial- or full-thickness diabetic foot ulcers treated with Biovance over a 12-week period.

In addition, time to complete wound closure and safety profile were monitored (Table 1).

MATERIALS AND METHODS

Fourteen patients with chronic nonhealing recalcitrant diabetic partial- or full-thickness foot ulcers were enrolled in the study. Ten patients were male, one of whom was enrolled as 2 and 14 on 2 separate occasions for 2 separate ulcers, and 2 were female. The patients ranged in age from 36 to 84 years old, with a mean age of 63.4 years. Consent was obtained voluntarily by each subject or their caretaker at the time of enrollment. A problem-focused history and physical examination were obtained initially, including duration of diabetes, concomitant diseases, medications, allergies, and complete history of the ulceration. Eligibility criteria were defined as type 1 or type 2 diabetes and initial wound measurement (1–49 cm^2) with extension through epidermal and dermal layers. The absence of infection was determined upon clinical evaluation. Adequate vascular status was established to be present in all patients through ankle brachial indices from 0.13 to 1.39 mm Hg (mean, 0.67 mm Hg) and transcutaneous peripheral oxygen pressure from 39.3 to 63.35 mm Hg (mean, 48.4 mm Hg). Wounds were graded 1 or 2 based on Wagner Classification. All wounds were granular with active bleeding of the wound bed and periphery before application of Biovance. Patients receiving corticosteroids or radiation, those with immunosuppresion, or patients who were undergoing treatment for collagen

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Table 1.

TIME TO WOUND CLOSURE AND SAFETY PROFILE

Patient	Age, y	Sex	Wound Thickness	Wagner Grade[8]	Ankle Brachial Indices	$TcPo_2$,* mm Hg	Wound Measurement, cm^3		No. Biovance Applications	Response (Group 1, 2, or 3)	Prior Treatments
							Pre	Post			
1	62	M	Full	2	0.13	46.3	9.67	27.3	3	Wound did not heal at 12 wk (group 3)	V.A.C., Apligraf, hydrocolloids, enzymatic debridements, chronic antibiotic treatments for resolved osteomyelitis
2	59	M	Partial	1	0.89	40.65	0.54	0.00	3	Wound healed at 8 wk (group 1)	Hyperbaric oxygen treatment, dry sterile dressings, saline dressings, debridements
3	71	M	Full	2	0.80	39	3.07		1	Patient removed from study (unevaluable)	Osteomyelitis, amputation right fifth toe, V.A.C. hyperbaric oxygen treatment, debridements
4	70	F	Full	2	0.63	46.75	0.96	0.00	2	Wound healed at 6 wk (group 1)	Amputation hallux; local wound care with bacitracin, dual inlay inserts with cutout impressions
5	71	M	Partial	1	0.58	63.35	0.49	0.05	3	Wound did not heal at 12 wk (group 2)	Application of silver sulfadiazine (Silvadene) and dry dressing
6	62	M	Full	2	Not performed	39.3	1.6	0.00	1	Wound healed at 5 wk (group 1)	Ulcer duration 1 y; repeated debridements and ictamil application
7	80	F	Full	2	1.39	44.13	1.0	0.00	3	Wound did not heal at 12 wk (group 3)	Enzymatic debridements, saline dressings
8	53	M	Full	1	1.39	40.04	10.94	3.4	3	Wound did not heal at 12 wk (group 2)	V.A.C., DARCO wedge for forefoot offloading
9	82	M	Full	2	Not performed	Not performed	4.5	Not performed	6	Protocol deviation, patient removed from study data	Application of Silvadene
10	54	M	Full	2	Not performed	45	55.0	Patient died	1	Patient died (unrelated to study) before study completion	Application of Silvadene with wet-to-dry dressing
11	36	M	Partial	1	Not performed	42.24	0.032	0.00	3	Wound healed at 9 wk (group 1)	Parafil and debridement
12	75	M	Full	2	0.91	Not performed	1.8	1.5	3	Wound did not heal at 12 wk (group 3)	Saline dressings
13	53	M	Full	2	Not performed	Not performed	0.7	Not available	3	Patient lost to follow-up at 6 wk	Saline dressings
14	59	M	Partial	1	0.97	40.65	0.19	0.9	3	Wound did not heal at 12 wk (group 3)	V.A.C., DARCO wedge for forefoot off-loading, Apligraf, hydrocolloids

Abbreviations: $TcPo_2$, transcutaneous partial pressure of oxygen; V.A.C., vacuum-assisted closure.
*Vascular laboratory values: Normal lower extremity $TcPo_2$ values exceed 40 mm Hg. Values below 30 mm Hg represent a risk of healing compromise, the degree of which increases as values decrease.

vascular disease or organ transplantation were excluded. Nutritional status was evaluated in all 13 patients based on albumin level averaging 3.3 mg/dL, 1 patient having had 2 separate wounds. Blood glucose was monitored weekly through a glucometer as well as hemoglobin A1C (HbA1C) averaging 7.7 mg/dL.

Osteomyelitis was present in 6 patients previous to enrollment and had been treated with intravenous antibiotic therapy,

surgical debridement, and tissue biopsy. Radiographs of the afflicted foot were obtained within 30 days of patient enrollment to ensure that the underlying osseous structures were not actively infected due to the chronicity of the ulcers. All necessary tracking information was recorded according to Food and Drug Administration (FDA) (21 CFR 1271.290). Approval to perform the study was obtained from the Scranton Institutional Review Board.

During the 12-week trial, all patients were given the option to voluntarily remove themselves from the study. All questions and concerns regarding serious adverse events, such as sensitivity to Biovance or any change in medical condition that could prevent the patient from continuing in the study (eg, disability or incapacity, inpatient hospitalization, life-threatening medical condition, or death), were discussed with the patients. All patients were aware that in previous patient trials, topical applications of amniotic membrane did not directly cause life-threatening conditions or result in death. They were also notified that if a patient developed signs of systemic or localized infection, they would be removed from the trial.

The patients' willingness and ability to comply with weekly office visits were assessed and determined to be not of significant concern or difficulty for any patient enrolled.

During the 12-week trial, each patient received up to 3 applications of Biovance followed by a secondary dressing per the primary authors of the study: Vaseline-impregnated gauze and wet gauze pads. The treatment for the upcoming week was determined after a weekly assessment by the researchers. If epithelialization had improved, Biovance was not applied, and the patient was instructed to apply a wet-to-moist dressing daily. If the wound had not epithelialized significantly, a second or third application of Biovance was performed. The patients were instructed to allow the collagen matrix and fibroblastic properties of Biovance to incorporate into the wound until the following week and to not remove the dressing. If systemic or localized signs of infection were present, the patient was told to call the office of the primary authors for clinical evaluation. If direct correlation of the active infection and the wound(s) was able to be determined, the patient was excluded from the study and treated according to the standard of care.

At the completion of the 12-week course, final assessment of the wound was performed and recorded. If the wound was taken to complete closure during the 12-week period, it was categorized as an excellent result (group 1). For those that responded to Biovance with a significant decrease in wound size of 50% to 80%, a fair result was noted (group 2). A wound was considered a failed response (group 3) if there was less than 50% improvement during the trial period (Table 2).

Table 2.

PATIENT RESPONSE CLASSIFICATION

Patient Response	Classification
100% Wound closure	Group 1
50 to 100% Wound closure	Group 2
<50% Wound closure	Group 3

RESULTS

Of the 13 patients initially enrolled in the study, 9 had completed the 12-week study without deviation. One patient had received Biovance on bilateral feet on separate occasions and therefore was enrolled as 2 separate subjects (2 and 14) with the approval of Celgene Cellular Therapeutics.

Of the 6 patients who were considered a failure, 2 patients (12 and 13) developed an infection that directly correlated with the diabetic foot ulcer being treated, and therefore those patients were removed from the study. Patient 13 was also lost to follow-up during the 12-week trial. One patient (9) received 6 applications of Biovance by physician request and was removed from the final data results. One patient (3) developed major complications of his ulceration, requiring above-the-knee amputation. Patient 10 received 1 application of Biovance and died during the 12-week trial due to medical comorbidities. This was reported to Celgene Cellular Therapeutics within 24 hours of the event coming to the authors' attention.

Of the 9 patients who were included in the final data, group 1 consisted of 5 patients (55.5%), group 2 consisted of 3 patients (33.3%), and group 3 consisted of 1 patient (11.1%). Although 3 patients in groups 1 and 2 (37.5%) were documented to have healed during the trial, their ulcers had continued to break down because of underlying osseous deformity. Patients 4 and 7 were taken to the operating room for osseous resection, planing, or foot and ankle reconstruction after their ulcers had either failed to respond or recurred. Bone biopsies were obtained from the prominent bone and did not show microbial growth. These patients are healed. Of the patients in group 3, patient 1 received local wound care from a home health agency that failed to adhere to treatment protocols while the Biovance was in place.

CASE REPORTS

Case 1

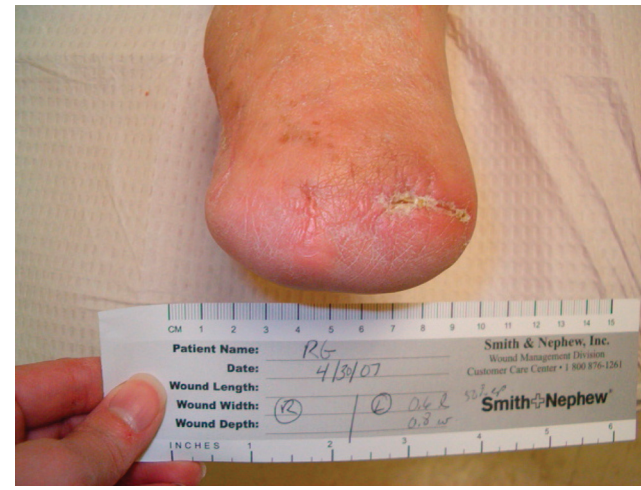
Patient 2 was a 59-year-old man with type 2 diabetes who presented to the office, requiring local wound care following hallux amputation secondary to osteomyelitis. The patient

previously underwent femoral popliteal bypass grafting in an attempt at limb salvage after multiple lesser-digit amputations. The patient's medical history included type 2 diabetes, hypertension, neuropathy, and lumbar disk disease. Current medications included hydrocodone/acetaminophen (Lorcet), methadone, amitriptyline, pregabalin (Lyrica), glipizide, and multivitamin. The patient had no known sensitivity to Biovance, nor displayed signs of sensitivity during the trial. Before enrollment, the patient received local wound care, including daily moist dressing changes and negative pressure therapy, surgical debridements, and allogenic dermal grafting. The partial-thickness grade 1 ulceration was at the distal stump of the hallucal amputation measuring 2.8 cm in length, 0.8 cm in width, and 2 mm in depth (0.44 cm^3).

At the time of enrollment, the transcutaneous oxygen pressure at the right transmetatarsal region was 40.65 mm Hg, and the ankle brachial index was 0.89 mm Hg. The patient's HbA_{1C} was recorded at 7.0 mg/dL, weekly blood glucose averaged 180 mg/dL, and albumin level was 4.2 mg/dL. Radiographs were evaluated and showed no signs of active or chronic osteomyelitis. Off-loading was maintained as the patient was being treated for bilateral foot ulcerations. The patient's ulceration was photographed weekly and recorded. Patient 2 received his initial application of Biovance, which was then covered by Vaseline-impregnated gauze and a wet-to-moist dressing. The patient maintained a clean dressing for 7 days without complication. At week 3, the patient's ulceration measured 2 cm in length, 0.2 cm in width, and 0.1 cm in depth (0.04 cm^3). Less than 50% epithelialization had occurred during this initial application; therefore, Biovance was applied a second time. The third application of Biovance was performed at week 4, as the wound measured 1.2 cm in length, 0.2 cm in width, and 2 mm in depth with 50% epithelialization and good quality of granulation. Upon presentation at week 8, the patient's ulceration completely healed (Figure 1).

Figure 1.

PATIENT 2: FOLLOWING 3 APPLICATIONS OF BIOVANCE WITHIN 8 WEEKS, WOUND COMPLETELY HEALED



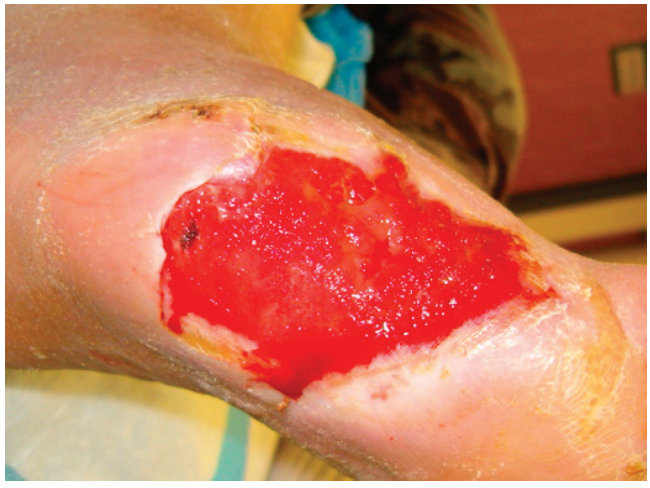
Case 2

Patient 8 was a 53-year-old man with type 1 diabetes who presented to the authors' office, requiring local wound care following incision and drainage with wide debridement, including a partial fifth-ray resection of the right foot because of gas gangrene. The patient's medical history included type 1 diabetes mellitus, anemia, thrombocytopenia, and hepatitis C. Current medications included glipizide 10 mg PO BID, spironolactone (Aldactone) 50 mg PO BID, metoprolol tartrate (Lopressor) 25 mg PO BID, and oxycodone hydrochloride and acetaminophen (Percocet) 5/325 PO every 4 to 6 hours. The patient was also receiving intravenous antibiotic therapy, including piperacillin/tazobactam 4.5 g intravenously every 8 hours and vancomycin 1 g intravenously every 12 hours, for an initial diagnosis of osteomyelitis that had since resolved. The patient had no known sensitivity to Biovance, nor displayed signs of sensitivity during the trial. Before enrollment, the patient received local wound care including daily moist dressing changes and negative pressure therapy. The full-thickness grade II ulceration was localized to the distal lateral right forefoot measuring 6.4 cm in length, 5.7 cm in width, and 3 mm in depth (10.94 cm^3) (Figure 2).

Per study guidelines, consent was obtained. All questions and concerns regarding the amniotic membrane and potential serious adverse events that could lead to withdrawal from the study were addressed with the patient.

Laboratory results were obtained and recorded. The transcutaneous oxygen pressure at the right transmetatarsal region was 40.04 mm Hg, and the ankle brachial index was 1.39 mm Hg. The toe brachial index was not obtained because of equipment failure. His HbA_{1C} was recorded at 5.8 mg/dL, weekly blood glucose averaged 114.6 mg/dL, and prealbumin level was 8.2 mg/dL. Radiographs were evaluated showing no signs of active or chronic osteomyelitis. Off-loading was maintained in a forefoot relief surgical shoe without complication. The patient's ulceration was photographed weekly and recorded. At week 1, patient 8 received his initial application of Biovance, covered by Vaseline-impregnated gauze and a wet-to-moist dressing. The patient maintained a clean dressing for 7 days without complication. Upon second evaluation, the patient's ulceration measured 6.4 cm in length, 6.1 cm in width, and 2 mm

Figure 2.
PATIENT 8: INITIAL WOUND FOLLOWING WIDE DEBRIDEMENT AND PARTIAL FIFTH-RAY AMPUTATION



in depth (7.8 cm^3). Less than 25% epithelialization had occurred during this initial week; therefore, Biovance was applied a second time. The third application of Biovance was performed at week 5, as the wound measured 5.2 cm in length, 4.1 cm in width, and 1 mm in depth with less than 50% epithelialization and good quality of granulation. At the completion of the 12-week trial, the patient's superficial ulceration measured 2 cm in length and 1.7 cm in width (3.4 cm^3), decreasing by 79%. Although patient 8 was included in group 2, the ulceration was clinically appropriate for split-thickness skin grafting at the completion of the trial (Figure 3).

DISCUSSION

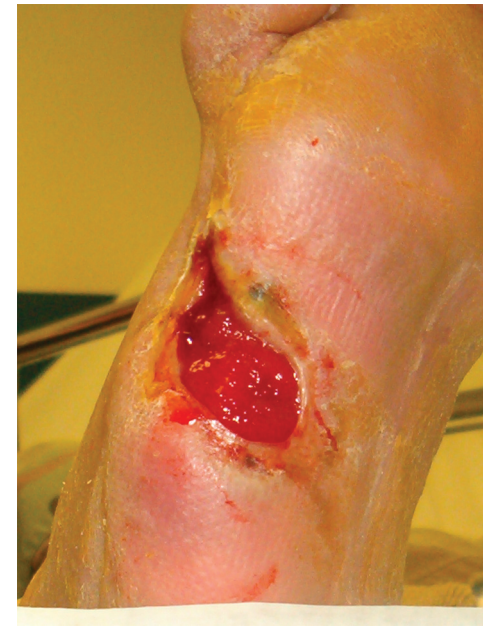
The concept of using amniotic membrane as a biological dressing has a history of implementation since the early 1900s. Stern and Sabella[5] hypothesized in their research that the ectodermal origin of the skin would be covered by "like" tissue; therefore, an immunogenic response would not occur. The chorion, in their experiments, was on the outer surface of the wound, firmly adhering to the paraffin that was placed as a wound covering. Following this work, various authors reported on the utilization of amnion and chorion membrane in such instances as forming an artificial vagina, treatment in the prevention of meningocerebral adhesion, and tendon repairs in fresh trauma.[6,7]

Douglas[8] performed research on chorioallantoic chick membranes where the investigator succeeded at grafting human membranes. She continued this research determining the potential for amniotic and chorionic membrane to function as an allograft. Douglas[8] proved that the chorionic membrane allowed for vascularization and re-epithelialization because of its collagen composition. The amniotic membrane is composed of a densely packed collagen matrix with a thickness of 6 to 8 cells, resulting in the tensile strength of this layer. Neovascularization would not occur at this level; however, because of the collagen and fibroblastic makeup, the allograft will "take" and incorporate to the wound.[6]

Trelford-Sauder et al[9] reported using amnion individually in cases of full-thickness wounds in vulvectomies and groin dissections. In this study, the amnionic membrane was placed in direct contact with the wound and remained in place until the wounds were taken to closure. In 14 days, re-epithelialization occurred to the wounds. Trelford–Sauder[9] also reported success in 1976 through experiments with sheep, where the histological healing process was analyzed. It was determined that closure of the surgical wounds occurred by epithelial in-growth and contracture of the scar formation occurred in those subjects with amniotic allograft. Nine of the lambs' surgically excised 5 × 5-cm squares healed; however, the control lambs that received local wound care with nitrofurazone (Furacin) dressing healed with a heavy, thickened scar.[9]

The preparation of amniotic membrane was studied by Ward and Bennett[10] in a study that compared tissue-cultured amnion,

Figure 3.
PATIENT 8: FOLLOWING 3 APPLICATIONS OF BIOVANCE; WOUND 79% DECREASE IN OVERALL SIZE



frozen and freshly prepared amnion, and lyophilized amnion. Tissue-cultured amnion was maintained in 37°C for a maximum of 3 weeks and maintained at a pH level of 7.4. The frozen amnion was rapidly frozen to −25°C and maintained at this temperature for 6 weeks. The warming process was 10 minutes at room temperature. Fresh amnion was stored at 4°C for 24 hours before usage.

Amniotic membrane that was lyophilized was freeze-dried at −30°C and irradiated with gamma rays. Before its use, the lyophilized amnion was immersed in Ringer's solution for 1 minute. These various preparations were tested on 27 patients, with a total of 40 chronic venous leg ulcers. The preparations were randomly paired with the leg ulcers. Of the 40 ulcers treated, 16 were treated with lyophilized amnion and resulted in 74.7% graft ''take'' compared with fresh and frozen amnion (56.7% and 53.3%). The overall time to complete closure averaged 26.45 days for the fresh and frozen allografts and 22.3 days for the lyophilized group.[10]

Biovance is a collagen-based decellularized, dehydrated topical wound covering produced from human amniotic membrane, the innermost layer of the amniotic sac. It is composed predominantly of collagen I, III, and IV as well as glycosaminoglycans, fibronectin, and laminin. The native collagen-based matrix is not disturbed during the dehydration process as well as the E-beam radiation process of sterilization. It is derived from the placentas of normal, full-term pregnancies of donors who are carefully screened medically and socially in accordance with the American Association of Blood Banks and the FDA. Because Biovance is of human derivation, it offers no immunogenicity, whereas the lyophilized amniotic membrane group in the study done by Ward and Bennett[10] revealed to have a higher ''take''; the process of dehydration and decellularization allows the practitioner easy accessibility and longer shelf-life with Biovance. Celgene Cellular Therapeutics reported on safety and biocompatibility of Biovance in nonclinical studies, of which ocular tissue of a rabbit, being more sensitive than human skin, was exposed to Biovance and offered no cytotoxicity, irritation, hemolysis, or pyrogenicity.

In conclusion, although complete wound closure was not achieved in all wounds enrolled in this study, the percentage (88.8%) of wounds that responded fairly was significant. The overall decrease in size for group 2 was 80% for 4 of the subjects. The primary objective of this trial was met by 4 patients healing within the 12-week period.

The secondary objective was met in all 14 wounds, as there were no adverse events associated with Biovance. The stipulations within this trial of 3 applications of Biovance within an allotted 12-week period were arbitrarily designed by Celgene Cellular Therapeutics. In the fair-results subset (group 2), it is of the authors' opinion that weekly applications of the amniotic membrane would have offered higher potential for complete closure and success for the trial. In studies such as those performed by Trelford and Trelford-Sauder,[5] Shun and Ramsey-Stewart,[11] and Eldad et al,[12] amniotic membrane was applied on a daily basis along with local wound care techniques for burns, fascial dermabrasion, chronic venous ulcerations, and decubitus ulcers. In comparison to the study performed by Kucan et al,[13] the application technique of Biovance is one that promotes reepithelialization in partial-thickness ulcerations. This emphasizes the results of this study, whereby 2 of the patients with complete closure had, in fact, partial-thickness ulcerations.

The historical background and current therapeutic utilization of amniotic membrane for the treatment of wounds offer a platform for future development of amniotic membrane to expedite wound healing in a safe and efficacious manner. Based on the results of this study, further clinical studies are warranted. ●

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