A Multicenter, Double-Blind, Placebo-Controlled Trial of Autologous Fibroblast Therapy for the Treatment of Nasolabial Fold Wrinkles

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BACKGROUND Changes associated with aging are partly due to loss of collagen and elastin. Treatment with autologous fibroblasts grown in culture (azficel-T) can help correct the appearance of aging by replacing lost dermal constituents.

OBJECTIVE To demonstrate the safety and effectiveness of autologous fibroblasts in the treatment of nasolabial fold (NLF) wrinkles.

METHODS AND MATERIALS Adults with moderate to very severe NLF wrinkles were randomized to receive three treatments with autologous fibroblasts or placebo at 5-week intervals. Blinded evaluators and subjects assessed efficacy using a validated wrinkle assessment scale.

RESULTS Three hundred seventy-two subjects were enrolled and underwent treatment. Seventy-eight percent of subjects treated with autologous fibroblast therapy and 48% of subjects treated with placebo achieved at least a 1-point improvement on the subject assessment at 6 months ($p < 0.001$), and 64% of subjects treated with autologous fibroblast therapy and 36% of those treated with placebo showed at least a 1–point improvement evaluator's assessment ($p < 0.001$). Adverse events were generally mild, and the treatment was well tolerated.

CONCLUSION Autologous fibroblast therapy is safe and effective for the treatment of NLF wrinkles. The availability of autologous cell therapy marks the beginning of a new phase in aesthetic therapy.

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Introduction

The demand for facial rejuvenation treatment around the world continues to grow.[1] Almost all current therapies are based on the implantation of foreign substances (e.g., cross-linked hyaluronic acid; bovine, porcine, or recombinant collagens; polymethylmethacrylate), the generation of wound-healing cascades through ablative skin resurfacing or chemical peeling, or ablation of existing structures such as telangiectasias or melanosomes. The ideal restorative agent would be nonforeign, minimally invasive, long lasting, and easy to administer.[2,3]

There is rapidly growing interest in cell therapies for treatment of many diseases and a broad belief that cell replacement may be more beneficial than other therapies.[4] With that interest has come new methodologies, capabilities, and techniques. We now have better cell culture techniques with which many nontransformed cell types can be expanded and maintained in culture for long periods. From

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these new techniques, a robust process for autologous cultured fibroblast (United States Adopted Name: azficel-T) growth has been developed and validated for reproducible results. Initial studies with these fibroblasts have demonstrated a good risk:benefit profile.[5] To fully demonstrate the safety and effectiveness of autologous fibroblast therapy for the treatment of nasolabial fold (NLF) wrinkles, two identically designed, placebo-controlled, multicenter, double-blind, parallel-group studies were performed. The results from the two studies are presented here as an integrated, single report.

Materials and Methods

An institutional review board (IRB) with jurisdiction over each investigative site approved the protocol. All subjects underwent an informed consent process and signed an informed consent form that the IRB had approved. The study was conducted in accordance with Good Clinical Practices and the principles that have their origins in the Declaration of Helsinki (revised Seoul, Korea, 2008).

Subjects could participate if they were aged 18 and older, expressed dissatisfaction with their NLF wrinkles based on a score of −1 or −2 on the Subject Wrinkle Assessment Scale (Table 1), and had bilateral moderate to severe NLF wrinkles as documented by a score of three or greater on the Evaluator Wrinkle Severity Assessment Scale (Table 2).

TABLE 1. Subject Wrinkle Assessment Scale

How do you feel about the wrinkles in the lower part of your face today?

Score	*Descriptor*
−2	I am very dissatisfied with the wrinkles of the lower part of my face.
−1	I am dissatisfied with the wrinkles of the lower part of my face.
0	I am somewhat satisfied with the wrinkles of the lower part of my face.
+1	I am satisfied with the wrinkles of the lower part of my face.
+2	I am very satisfied with the wrinkles of the lower part of my face.

TABLE 2. Evaluator Wrinkle Severity Assessment Scale[6]

Score	*Descriptor*
0	No wrinkle visible
1	Just perceptible wrinkle
2	Shallow wrinkle
3	Moderately deep wrinkle (definite and distinct wrinkle)
4	Deep wrinkle, well-defined edge (prominent wrinkle, well defined edge)
5	Very deep wrinkle, redundant fold (very severe wrinkle, pronounced edge)

Subjects were ineligible if they had excessively redundant skin in the treatment area or wrinkles longer than 20 cm in total that could not be lessened by physically spreading the skin. In addition, subjects were excluded from the study if they had a history of autoimmune disorders, organ transplantation, cancer not in remission, active or chronic skin disease, a genetic disorder involving fibroblasts or collagen such as epidermolysis bullosa or ataxia-telangiectasia, or a history of basal cell carcinoma; were pregnant or breast-feeding; had previously received autologous fibroblast treatment; had undergone any confounding therapy in the lower two-thirds of the face within 1 year or any investigational treatment within 30 days; or had an allergy to collagen, bovine products, local anesthetics, gentamicin, or amphotericin B.

After undergoing informed consent and confirmation of eligibility, subjects were randomized 1:1 to receive autologous fibroblasts in suspension or a placebo of Dulbecco's modified Eagle medium (DMEM) without phenol red. Subjects underwent three 3-mm punch biopsies in one retroauricular area to harvest donor fibroblasts. Donor sites were closed with sutures or adhesive bandages or left open to heal by secondary intention at the discretion of the investigator. Donor material was processed as detailed below. Upon receipt of expanded fibroblast suspensions, subjects underwent injections of suspended cells or vehicle to the right and left NLFs in three sessions at 5-week intervals.

Follow-up visits were conducted 2, 4, and 6 months after their final injections.

Efficacy and Safety Endpoints

Efficacy was assessed using four parameters: evaluation of live subject appearance by blinded evaluators, self-evaluation of subject appearance, evaluation of final improvement by blinded evaluators using photographs, and self-evaluation of final improvement by subjects using photographs. Blinded evaluators (dermatologists or plastic surgeons) unaware of the subjects' treatment assignment viewed the subjects at each efficacy assessment and scored each NLF wrinkle using the validated Evaluator Wrinkle Severity Assessment Scale.[6] These scores are a static assessment of wrinkle severity at a single point in time, not a comparison to prior scores or photographs. Similarly, subjects performed their own assessments using a Subject Wrinkle Assessment Scale.[6] These assessments were performed just before the third injections and 2, 4, and 6 months after the final injections. Standardized photographic equipment was used to ensure that reproducible photographs were captured at each visit. At the final study visit (6 months after the final injection), the blinded evaluators and subjects compared the baseline, follow-up, and final photographs using the Impression of Change from Baseline Scale (Table 3).

The primary analysis was performed on all randomized subjects who received at least one treatment (treating both NLF wrinkles). Subjects with missing data were treated as treatment failures for data analysis. Success was defined as 1- or 2-point improvement on the Subject Wrinkle Assessment Scale and Evaluator Wrinkle Severity Assessment Scale for both NLF wrinkles measured 6 months after the final treatment. The Fisher exact test was used for all categorical variables in performing analyses of efficacy.

Adverse events (AEs) were collected at each visit and interpreted by the investigator with respect to their relatedness to the treatment.

Fibroblast Culture

After biopsy collection, skin samples were submerged in phosphate buffered saline (PBS), and shipped overnight at 2–8°C to the sponsor facility for processing. Once received at the sponsor facility, biopsy samples were inspected and transferred to manufacturing for processing.

All cell processing occurred under aseptic conditions (ISO 5, 209E Class 100) within a biological safety cabinet. Manufacturing activities occurred under Current Good Manufacturing Practices. After an antibiotic wash containing gentamicin and amphotericin B, biopsy tissue was subjected to enzymatic dissociation in a collagenase enzyme cocktail at 37°C (Liberase Blendzyme, Roche, Penzberg, Germany).

After the dissociation was complete, cells were seeded into a T-175 culture flask within Iscove's modified Dulbecco's medium with phenol red (IMDM) supplemented with antibiotics and fetal bovine serum (FBS). Culture flasks were stored in a humidified incubator at 37°C with 5% carbon dioxide. Routine feeding of the fibroblasts was performed using IMDM with FBS by removing half of

TABLE 3. Evaluator and Subject Impression of Change from Baseline

Score	*Evaluator*	*Subject*
−2	Wrinkle is much worse than before	Appearance is much worse than before
−1	Wrinkle is worse than before	Appearance is worse than before
0	Wrinkle is the same as before	Appearance is the same as before
+1	Wrinkle is better than before	Appearance is better than before
+2	Wrinkle is much better than before	Appearance is much better than before

the volume of spent medium and adding back the volume with fresh medium. Once the culture reached preestablished confluence specifications, cells were rinsed with PBS and exposed to trypsin–ethylenediaminetetraacetic acid to remove cells from the surface of the flask. Cells were passaged to a T-500 triple culture flask and fed on a routine basis.

After confluence was achieved in the T-500 flask, cells were rinsed and passaged using trypsinization as previously described into a 10-layer cell stack and fed on a routine basis. After reconfluence in this cell stack, fibroblasts were harvested and cryopreserved in IMDM and freezing medium (Profreeze, Lonza, Walkersville, MD) supplemented with dimethyl sulfoxide. Cells were stored in the vapor phase of liquid nitrogen. No serum is present in the cryopreservative formulation. After cryopreservation, a series of release tests were performed, including efficacy analyses confirming the cell population count, 85% or greater viability, and identity of the overall population as 98% or more fibroblasts. Additional tests for sterility, *Mycoplasma* sp., and endotoxin contamination were performed.

All cells were cryopreserved and removed for use in injection preparation as needed. Culture was not continuous during the injection schedule timeframe. If additional cells were required after cryopreservation of the primary culture, a frozen vial was removed and used to reseed a cell stack. Cells were fed and harvested as previously described. In total, fibroblasts used for injection were cultured for approximately two to five passages.

Before use, the cells were thawed, washed with PBS and DMEM, resuspended at a concentration of 1.0–2.0 $\times$ 10^7 cells/mL, and shipped overnight at 2–8°C to the treatment center for administration the next day. Before shipment, a series of additional efficacy release tests were performed on the final product, including confirmation of cell count and assessment of cell viability. In addition, safety tests, including Gram stain, endotoxin detection, and sterility, were performed on each released injection set in the series. Before use at the treatment center, the cell suspension was stored at 2–8°C and then allowed to warm to room temperature before use. Sites received blinded shipments of cell suspension or placebo.

Injection Technique

Anesthesia was provided at the discretion of the investigator in the form of topical, local infiltrative, or regional blocks using customary local anesthetic agents. The area of treatment was prepared with an antiseptic before injections. Cell suspension or placebo was injected using a 29- or 30-G needle in a retrograde threading technique directly over the NLF wrinkle. A total of 0.1 mL of cell suspension was injected along each 1 cm of wrinkle. The material was placed into the superficial papillary dermis and confirmed by the appearance of blanching and wheal formation at the site of each injection. The same amount of material was injected at each session. No massage or other manipulation of the areas was performed. Subjects were advised to avoid the use of soaps, cosmetics, or any other products to the face for 72 hours after each injection session. After injections, limited short-term indirect application of ice to the treatment area was allowed at the discretion of the investigator.

Results

Four hundred twenty-one individuals signed an informed consent form and were enrolled in the study at 13 centers across the United States. Of these, 372 underwent at least one treatment session with study material. Of those who enrolled but did not receive treatment, 19 were discontinued because of difficulties with the biopsy specimen or manufacturing considerations, 15 withdrew consent, three were lost to follow-up, two had AEs that precluded their participation and seven were discontinued for miscellaneous reasons. The remaining 372 subjects are the modified intention-

to-treat population upon which safety and efficacy analyses were performed. Three hundred forty-nine (94%) subjects completed all injections and follow-up visits. The reasons for discontinuation after initiating injections include five subjects who withdrew consent, four who were nonadherent to the protocol, two who withdrew because of AEs, one who was dropped at the sponsor's request, and one who was lost to follow-up. The demographics of the active and placebo groups are shown in Table 4.

The proportion of subjects achieving 2-point improvement in the blinded evaluator scores for right and left NLF wrinkles is shown in Figure 1A. A statistically significant difference between the active and placebo groups was found at the first assessment (and final injection visit) approximately 10 weeks after the first injections. This difference remained or increased throughout the 6-month follow-up period. Subjects' self-assessment using the Wrinkle Assessment Scale is shown in Figure 1B and is similar to the results seen for the evaluator scores. As might be expected, subject grading was generally more positive than the evaluator scores. When a 1-point improvement for both NLFs is applied, a significantly greater proportion of subjects showed such improvement, as displayed in Figure 2. Again, the results show improvement as early as the third injection session and persist through the end of the follow-up period. The differences at each time point for 1- and 2-point improvement efficacy criteria are statistically significant ($p < 0.001$). Photographs for subjects treated with autologous fibroblasts are shown in Figure 3.

TABLE 4. Subject Demographic Characteristics

Characteristic	*Autologous Fibroblast Therapy (n = 181)*	*Placebo (n = 191)*
Age, average (range)	55.9 (23–76)	55.5 (27–79)
Age, n (%)		
<50	40 (22)	52 (27)
50–64	112 (62)	111 (58)
≥65	29 (16)	28 (15)
Sex, n (%)		
Female	165 (91)	174 (91)
Male	16 (9)	17 (9)
Race, n (%)		
Caucasian	156 (86)	169 (88)
Hispanic	20 (11)	18 (9)
African American	2 (1)	3 (2)
Asian	2 (1)	0
American Indian or Alaska native	0	1 (<1)
Other	1 (<1)	0

There was no statistically significant difference in race, sex, or age between the autologous fibroblast therapy and placebo-treated groups.

The evaluator assessment of impression of change from baseline in wrinkle appearance using comparison of photographs and the Impression of Change from Baseline Scale shows that 50% of the subjects in the treatment group and 18% in the placebo group ($p < 0.001$) were responders (graded as better or much better). Subject self-assessment using the same scale resulted in 58% response in the treatment group and 23% response in the placebo group ($p < 0.001$).

Safety

A total of 1,076 treatment visits were conducted, an average of 2.8 per subject in the autologous fibroblast therapy group and 3.0 in the placebo group. Ninety-three percent of subjects underwent all three treatment sessions. The incidence of AEs was similar in the two treatment groups; 113 subjects in the autologous fibroblast therapy group (62%) and 113 in the placebo group (59%) sustained at least one AE. The incidence of AEs is shown according to frequency in Table 5. The most common AEs reported were redness, swelling, and bruising in and around the injection sites. Redness was more common in patients who received autologous fibroblasts, and bruising was more common in patients receiving the placebo. No other AEs were found to be significantly different in incidence between the autologous fibroblast and placebo groups. The majority of AEs were mild to moderate in severity and felt to be related to the

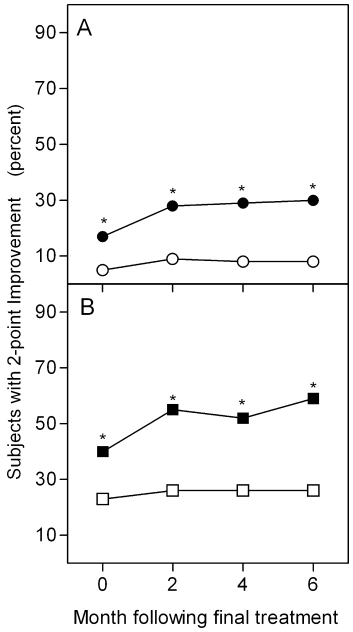

Figure 1. The percentages of subjects with a 2-point improvement based on the investigator assessment (A) are shown for subjects treated with autologous fibroblast therapy [filled squares] and placebo [open squares]. Subjects with a 2-point improvement based on subject assessment are shown in (B). An asterisk (*) indicates a statistically significant difference between autologous fibroblast therapy and control treatment based on the Fisher exact test ($p < 0.05$).

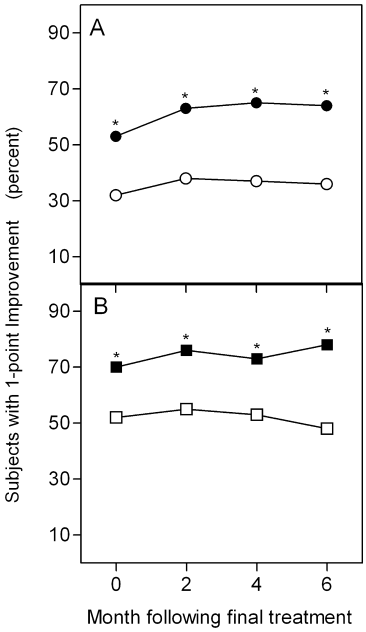

Figure 2. The percentages of subjects with a 1-point improvement based on the investigator assessment (A) are shown for subjects treated with autologous fibroblast therapy [filled squares] and placebo [open squares]. Subjects with a 1-point improvement based on subject assessment are shown in (B). An asterisk (*) indicates a statistically significant difference between autologous fibroblast therapy and placebo treatment based on the Fisher exact test ($p < 0.05$).

injection process. Two subjects in the autologous fibroblast therapy group discontinued the study because of AEs: one for moderate injection site pain and the other for mild injection site bruising. Treatment-related AEs resolved within 1 week of treatment in 94% of subjects who experienced them; there was no difference between the autologous fibroblast therapy and placebo-treated subjects with regard to duration of AEs. Only six AEs in the autologous fibroblast therapy treatment area lasted longer than 14 days: two cases of papules, two of hyperpigmentation, and one each of injection site redness and irritation. There were no treatment-related AEs other than those in the area of injection.

Seventeen serious AEs were noted during the study —nine in the autologous fibroblast therapy group and eight in the placebo group. None of the events were considered to be related to study treatment. A single death occurred in the study. This subject enrolled, had a biopsy sample collected, and died of unrelated causes before receiving study treatment.

Four subjects reported developing nodules in the treatment area: two each in the treatment and placebo groups. All nodules resolved within 72 hours, and none required treatment. No cases of localized or generalized hypersensitivity to the treatment were reported. A single subject randomized to autologous fibroblast therapy developed a basal cell carcinoma near the treatment area 5 months after the last injections. The lesion was considered to be unrelated to autologous fibroblast therapy treatment and was treated with excision, and the subject completed the study. No recurrences have been noted in follow-up. No significant

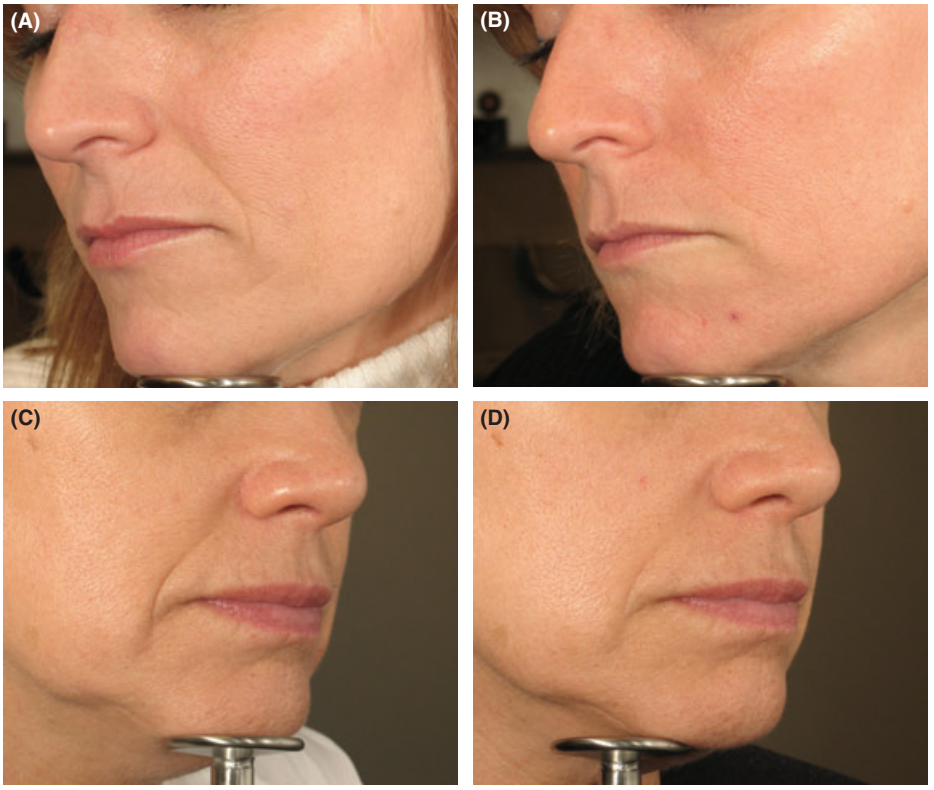

Figure 3. Subjects underwent three treatment sessions. (A) Baseline (pretreatment) image; (B) 6-months after final study treatment, 2-point improvement; (C) baseline (pretreatment) image; (D) 6 months after final study treatment, 2 point improvement.

differences in safety were found according to race, sex, or age.

Discussion

Autologous fibroblast therapy is the first cell therapy for aesthetic improvement to show statistically significant benefit in large blinded controlled trials. Physicians who use and compare various aesthetic drugs, devices, and biologic agents should note that this study is far larger and more comprehensive and included stricter endpoints for success than studies of most other aesthetic therapies. Most studies of devices or procedures to treat the NLF area are bilateral designs wherein each subject serves as a control, and the novel treatment must be comparable with an existing treatment.[7–14] Autologous fibroblast therapy is regulated as a cell therapy, so to ensure the safety and efficacy of this novel product, a parallel design was chosen to assess this autologous fibroblast therapy. This resulted in two large, well-powered studies that demonstrated clinical benefit to a high degree of statistical significance.

The timeline for wrinkle improvement is different for this product than for most dermal fillers. It is expected that the product results in the deposition of new collagen but does not use direct volume replacement. Consequently, it has a more gradual onset of effect than the immediate correction seen with dermal fillers. It is encouraging to note that statistically significant differences were seen as early as 2 months after the start of treatment and before the injection series was completed, although most exciting is the shape of the curve from follow-up months 2 through 6. Unlike most dermal filler products, Autologous fibroblast therapy benefit showed no signs of degradation during the follow-up period without reinjection.[15–17] Prior

TABLE 5. Treatment-Emergent Adverse Events Occurring in at Least 2% of Subjects in Either Treatment Group

	n *(%)*	
Adverse Event	*Autologous Fibroblast Therapy (n = 181)*	*Placebo (n = 191)*
Injection site redness	37 (20)	23 (12)
Injection site bruising	9 (5)	25 (13)
Injection site swelling	21 (12)	15 (8)
Injection site bleeding	10 (6)	15 (8)
Injection site pain/irritation	12 (7)	6 (3)
Upper respiratory tract infection	10 (6)	8 (4)
Sinusitis	4 (2)	10 (5)
Injection site papules	6 (3)	3 (2)
Nasopharyngitis	6 (3)	4 (2)
Arthralgia	5 (3)	4 (2)
Nausea	3 (2)	3 (2)
Headache	1 (<1)	6 (3)

studies of autologous fibroblasts showed continued benefit 1 year after treatment.[5] It could be expected that the correction noted would persist considerably longer than has been seen with many existing therapies. How long the results will last is not yet known and represents a topic for further research. In addition, the results demonstrated in this trial are similar to those seen with dermal fillers when similar endpoints are compared. The average improvement in the Evaluator Wrinkle Severity Scale score for a recent dermal filler study was 1.3 out of 5 (26%), versus 1.3 out of 6 (22%) for autologous fibroblast therapy in this study.[8]

This study targeted the treatment of NLF wrinkles. The improvement seen is in wrinkles, not in folds. Like with any aesthetic procedure, good patient selection and management of expectations are critical to successful outcomes. The data from the Subject Change from Baseline and Subject Wrinkle Assessment scores show considerable perception of benefit and satisfaction. It is reassuring to physicians when changes noted on evaluator scores are meaningful to patients when they assess their results.

This study is the culmination of more than a decade of laboratory and clinical research work. After the process for fibroblast replication was perfected, the product became commercially available in the United States and elsewhere without regulatory oversight. Subsequently, it was determined that the product should fall under purview of the Food and Drug Administration. The product was removed from the commercial marketplace, and a series of studies was undertaken to demonstrate the safety and effectiveness of the treatment. Prior studies focused on multiple facial areas and ideal dosing concentrations and frequencies. Through that early clinical work, the current treatment regimen of three administration sessions, each 5 weeks apart, with a dose of 0.1 mL of a suspension of 1.0 to 2.0 $\times$ 10^7 cells/mL was determined to be ideal.

The exact mechanism of action of injected autologous fibroblasts remains unknown. They may exert their effect through one of several mechanisms. These could include the direct secretion of increased amounts of collagen and elastin, the induced proliferation of native fibroblasts, the secretion of cofactors that otherwise augment the dermal milieu, or simply multiplication of the transplanted fibroblasts. Most likely is it a combination of several of these processes.[18–21] A study of the histologic appearance of skin treated with autologous fibroblasts has recently been completed, and the results will be presented in a subsequent article. Once the process is better understood, it is likely that additional refinements can be made to further improve the clinical response.

Safety in aesthetic therapy is always of paramount concern. The AE profile of autologous fibroblast therapy treatments is as good as or better than those of many of the existing therapies.[22–25] The local findings of erythema, bruising, and swelling in the treatment area are not unexpected and have

a short overall duration. No hypersensitivity reactions were noted, and no long-term nodules or other local problems were seen. A theoretical risk of the enhancement of malignancies with autologous cell therapy has been raised.[26,27] These studies, although not powered to capture rare AEs, showed just one cutaneous malignancy near the treatment area. Given the number of subjects and duration of the trial, the incidence of cutaneous malignancy is consistent with background rates.[28] Although the studies were open to all potential subjects, the number of Asian and African-American subjects was not representative of the U.S. population. Given the nature of the therapy, it is unexpected that these racial groups would have significantly different risks than the study population as a whole, although physicians may want to proceed more cautiously when using autologous fibroblast therapy in these groups.

Conclusion

Autologous fibroblast therapy therapy is the first natural, biologic treatment modality in aesthetic medicine. The treatment is safe, effective, and easy to administer. The opportunity to replace lost or atrophied tissue with autologous material adds a significant option for physicians and patients. The concept of receiving their own cells, having a natural correction, and the likelihood of persistence will be attractive features to many patients. Physicians will now be able to offer patients a personalized option for NLF wrinkles. Given the unique characteristics of autologous fibroblast therapy, the range of potential applications for this novel therapy is extensive. We can look forward to clinical verification of other uses for this therapy and further expansion of the utility of autologous fibroblasts in general and aesthetic medicine.

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