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Prospective trial of Adipose-Derived Regenerative Cell (ADRC)-enriched fat grafting for partial mastectomy defects: The RESTORE-2 trial

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Abstract

Aims: Women undergoing breast conservation therapy (BCT) for breast cancer are often left with contour defects and few acceptable reconstructive options. RESTORE-2 is the first prospective clinical trial using autologous adipose-derived regenerative cell (ADRC)-enriched fat grafting for reconstruction of such defects. This single-arm, prospective, multi-center clinical trial enrolled 71 patients post-BCT with defects $\leq$150 mL.

Methods: Adipose tissue was collected via syringe lipoharvest and then processed during the same surgical procedure using a closed automated system that isolates ADRCs and prepares an ADRC-enriched fat graft for immediate re-implantation. ADRC-enriched fat graft injections were performed in a fan-shaped pattern to prevent pooling of the injected fat. Overall procedure times were less than 4 h. The RESTORE-2 protocol allowed for up to two treatment sessions and 24 patients elected to undergo a second procedure following the six month follow-up visit.

Results: Of the 67 patients treated, 50 reported satisfaction with treatment results through 12 months. Using the same metric, investigators reported satisfaction with 57 out of 67 patients. Independent radiographic core laboratory assessment reported improvement in the breast contour of 54 out of 65 patients based on blinded assessment of MRI sequence. There were no serious adverse events associated with the ADRC-enriched fat graft injection procedure. There were no reported local cancer recurrences. Injection site cysts were reported as adverse events in ten patients.

Conclusion: This prospective trial demonstrates the safety and efficacy of the treatment of BCT defects utilizing ADRC-enriched fat grafts.

Keywords: Mammaplasty [E04.680.500]; Transplantation; Autologous [E04.936.664]; Mesenchymal stem cell transplantation [E04.936.225.687.625]; Carcinoma; Ductal; Breast [C04.588.180.390]; Mastectomy; Segmental [E04.466.701]; Subcutaneous fat [A10.165.114.830.750]

Introduction

Breast conservation therapy (BCT) commonly results in irregular soft tissue defects that are challenging to reconstruct, particularly in patients who have had adjunctive radiotherapy. These patients often have few options for reconstruction because commercially available synthetic implants are not suitable for partial breast reconstruction, while flap procedures can be associated with significant morbidity in addition to considerable cost.[1] Given these challenges, surgeons continue to look for novel reconstructive options for BCT patients. There is an increasing body of evidence demonstrating that autologous fat grafting may be useful in the treatment of breast defects

Abbreviations: BCT, breast conservation therapy; ADRC, adipose-derived regenerative cells.

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however, there is great variability among surgeons in the methods of graft harvest, processing, delivery and outcome measurement.[2] In addition, clinical outcomes frequently show unpredictable graft survival and inconsistent results.[3]

Optimization of fat grafting outcomes necessitates standardization of the procedural steps and the potential use of additives to encourage graft survival. It is now known that subcutaneous fat is a rich source of stem and regenerative cells.[4] Studies have shown that supplementing fat grafts with these cells, termed Adipose-Derived Regenerative Cells (ADRCs), results in significantly increased fat graft retention in small animal models.[5,6] One recent comparative clinical study of fat grafting to the face showed that high levels of patient satisfaction were achieved with fewer procedures using cell-enriched fat grafts than with grafts performed without supplemental ADRCs.[7] This response appears primarily due to transient secretion of growth factors that promote normal angiogenesis, decrease apoptosis, and modulate the immune response (Toyoda et al., manuscript submitted).[8] The regenerative cells in adipose tissue are so abundant that the need for culture expansion to reach a therapeutic dose is eliminated.[9] Thus, a patient's adipose tissue can be harvested, processed (in part to extract ADRCs) and reinjected back into the patient during the same surgical procedure.

Preclinical work, as well as an emerging body of clinical data, suggests that ADRCs may improve graft survival and aid wound healing.[10–12] RESTORE-2 investigates the use of ADRC-enriched fat grafting as a novel reconstructive option for BCT patients. This is the first prospective clinical trial in this patient population to evaluate the feasibility and efficacy of this technique which integrates regenerative medicine and reconstructive surgery.

Materials and methods

RESTORE-2 is a prospective, single-arm, multi-center clinical trial which evaluated the use of ADRC-enriched fat grafting for breast reconstruction post-BCT. Seven clinical sites participated within the European Union including: Spain (2), United Kingdom (2), Belgium (2), and Italy (1). Ethics Committee approval was obtained at each institution and all patients provided written informed consent.

Co-primary endpoints included (1) patient and investigator satisfaction with functional and cosmetic results and (2) improvement in overall breast deformity at 12 months post-index procedure. Secondary endpoints consisted of improvement in breast volume and shape, skin pigmentation abnormalities, overall breast deformity and quality of life. In addition, adverse event profile, resource utilization and number of treatments required were evaluated.

Patient selection

Female patients (18–75 years of age) presenting with partial mastectomy defects up to 150 mL and without breast prosthesis, were eligible for enrollment. Those with a history of breast carcinoma; tumor classification up to T2-N0M0, tumor size $\leq$3 cm at time of excision, and $\geq$12 months since the last cancer treatment were invited to participate. Key inclusion criteria included (1) the ability to undergo liposuction for graft acquisition, (2) presence of mild breast damage post-BCT, (3) minimum of 1 cm between skin and chest wall, (4) a minimum of 2/3 of the breast remaining after BCT, and (4) no continuous adhesion of skin to bone >3 cm. Major exclusion criteria were (1) history of autoimmune disorder, (2) connective, metabolic, or atrophic skin disease, (3) history of keloid scarring or other malignancy, (4) chronic anticoagulant therapy, and (5) Body Mass Index (BMI) >30.

Treatment

Adipose tissue harvesting

The procedure was performed under general or local anesthesia as determined by physician preference, patient preference, and facility capabilities. Prior to harvest, the volume of the defect and required graft were visually estimated by the surgeon. Approximately twice the intended volume of the graft was harvested using standard tumescent, syringe-based liposuction under general or local anesthesia. Adipose tissue was subsequently divided into two equal fractions, one for the extraction of ADRCs and the other for use as the fat graft.

Preparation of the ADRC-enriched fat graft

One fraction of the lipoaspirate was added to the Celution® system (Cytori Therapeutics; San Diego, CA) where, with the addition of a proteolytic enzyme reagent (Celase®, Cytori Therapeutics; San Diego, CA), the ADRCs were released from their bound matrix, washed to remove residual enzyme, and then concentrated within the closed automated system in the operating room. Lin *et al.* characterized Celution output from 6 patients as 2.95×10^5 stromal vascular cells per mL of lipoaspirate, with a mean of 86.6% viability.[13] They further identified relative frequencies of the major populations using flow cytometry. Upon completion of this process (approximately 90 min), the suspension of ADRCs ($\sim$5 mL) was retrieved from the Celution system using an 18-gauge spinal needle. The second fraction of adipose tissue was then added to Celution system where it was washed with Lactated Ringers solution using gravity sedimentation/floatation. The concentrated ADRCs were then added to the washed graft tissue in the system and mixed to create the ADRC-enriched fat graft. Following the completion of tissue processing, the ADRC-enriched fat graft was aseptically transferred to the sterile field using 60 mL Toomey syringes. Centrifugation (1500 rpm/5 mins) of fat grafts prepared with this approach has shown

that adipose tissue comprises approximately 65% of the graft volume, the remainder of which is washing fluid.

Delivery of the ADRC-enriched fat graft

Three to four 2 mm stab incisions were made outside the region of the breast defect. Pre-tunneling was first performed with a blunt cannula to release scar tissue and define the tissue planes for injection. Injections were performed using the Celbrush® (Cytori Therapeutics; San Diego, CA), which consists of a stainless steel thumb-controlled syringe adapter designed to provide micro-droplet dispersion of graft. Graft delivery was accomplished with injections being performed in a fan-shaped pattern in different trajectories to maximize the surface area of the graft to native tissue. After graft delivery, the patient was placed in the sitting position to examine the results and additional scar release and/or graft injections were performed as necessary.

Post-operative care

Wounds were closed with steri-strips followed by dry gauze dressing and supportive brazier. Patients were either discharged from the hospital after recovery from anesthesia, or were kept in hospital overnight for observation. All patients were seen at 3–7 days post-treatment for wound check and assessment.

Assessments

General assessments included medical history and physical exam, cosmetic glandular and cutaneous sequelae as described by Clough et al.,[14] LENT-SOMA scale for breast carcinoma radiotherapy,[15] and Quality of Life (QOL) as assessed by SF-36v2[16] (Quality Metric; Lincoln, RI). Clough's classification system for post-BCT breast deformities subjectively evaluates overall breast deformity, symmetry between breasts, appearance of scarring, skin pigmentation, and overall satisfaction with treatment results. Using these metrics, patient and investigator satisfaction were assessed using the following six-point Likert Scale[17]: extremely dissatisfied, very dissatisfied, dissatisfied, satisfied, very satisfied, and extremely satisfied. The Late Effects Normal Tissues (LENT)-Subjective Objective Management Analysis (SOMA) scoring system addresses both the patient's perception of her physical symptoms as well as the physician's opinion of symptom morbidity using a scale of zero to four; four being maximally symptomatic.

Mammograms were obtained within 30 days prior to treatment to rule out malignancy and subsequently obtained according to standard of care. Resource utilization, duration of stay, and days off work were also captured. All adverse events were collected, evaluated and reported as required until resolution or completion of the trial. A second treatment was permitted at the six month time-point if the patient so desired.

MRI assessment

T1-weighted MRI images were collected at baseline, six and 12 months post-index treatment and were evaluated by an independent core lab (BioClinica; Lyon, FR) blinded to image sequence. Early in the study, the MRI Assessment Protocol required amending when it was found that MRI was not sensitive in determining breast volume and detecting defects in the superior and lateral periphery of the breast. Therefore, additional qualitative assessments of changes in breast contour and defect shape were implemented. All baseline, six and 12 month MRI images were scored by two independent radiologists blinded to patient sequence using a five-point Likert Scale for two variables, breast defect and breast contour.

Statistics

Independent data management and statistical analysis were performed by Synteract, Inc. (Carlsbad, CA). Continuous variables were summarized with means, standard deviations, medians, minimums, and maximums. *P*-values were based on a paired *t*-test change from baseline comparison. A response was categorized as "satisfied" for any answer with a Likert score of 4, 5 or 6 (satisfied, very satisfied or extremely satisfied). All analyses and tabulations were performed using SAS® (SAS Institute, Inc.; Cary, NC) Version 8.2 or higher on a PC platform. Evaluable patients were defined as those patients who completed the index procedure and a minimum of the six or 12 month follow-up visit assessments.

Results

Patient selection

A total of 71 patients were enrolled and 67 patients completed follow-up according to protocol. Two patients were lost to follow-up. One was excluded due to inability to complete graft preparation according to protocol and the other withdrew informed consent at one week post-treatment. In one patient both breasts were treated. Therefore, a total of 68 breasts were included in the analysis.

Patient demographics

Median age at the time of treatment was 52 years (range 37–68 years). Median BMI at baseline was 24.5 (range 17–31) and did not change significantly over the course of the study. The median defect volume was 100 mL (range 35–150) as visually estimated by investigators. It was reported that 61 patients underwent radiation therapy as part of BCT treatment. Two patients did not receive

Table 1
Details of breast cancer treatment and tumor characteristics.

	Median (Range)
Months since breast cancer diagnosis	51.6 (17.4–249.5)
Months since last radiation treatment	45.4 (11.1–239.5)
Cumulative radiation dose (Gy)	60 (12–66)
Tumor size (cm width) at time of excision	1.5 (0.4–4)
Weight (gm) of excised specimen	46 (4.9–275)

radiation and the status of radiation treatment was not known for another 4 patients. Further details regarding tumor characteristics, radiation history, and time course are summarized in Table 1.

Results of surgical treatment

General anesthesia was used in 65 of the 67 primary lipoaspiration procedures; one patient underwent local anesthesia and one patient underwent regional anesthesia. Following the index treatment, an optional second treatment at six months was permitted at the discretion of the patient and/or investigator to optimize the final cosmetic result. Of the 67 evaluable patients, 24 patients underwent a second procedure representing a procedural rate of 1.35 procedures per patient. All patients who had a second procedure underwent general anesthesia. Details regarding time requirements and tissue volumes for each procedural step can be found in Table 2.

Assessments

The LENT-SOMA scale included investigator and patient assessment of post-radiation signs and symptoms. The investigators of the trial found that LENT-SOMA was insufficiently sensitive to adequately reflect the clinical improvements seen in this patient population. Patients with LENT-SOMA III and IV scores (most severe symptoms) were excluded during screening, which may have contributed to the subtle LENT-SOMA score changes observed in the trial. Nonetheless, the investigators reported improvement from baseline through 12 months in the degree of retraction or atrophy in 29 out of 67 patients, while 34 patients had no change and 4 patients reported worse symptoms. Post-radiation fibrosis at 12 months was reported as improved in 29 patients, while 35 patients had no change and 3 patients had worse symptoms. Finally, management of atrophy was reported as improved in 17 patients, with 48 patients having no change and 2 patients reporting worse symptoms. Improvement in these three measures reached statistical significance.

The cosmetic glandular and cutaneous sequelae survey demonstrated mean improvement or stability from baseline through 12 months on all measures. For example, improvement in degree of fibrosis and deformity was observed in 35 patients, while 30 patients had no change and 2 patients reported worse symptoms. Breast asymmetry improved from baseline through 12 months in 36 patients, while 29 patients had no change and 2 patient reported worse symptoms. Similarly, hypertrophic or retractile scars improved in 40 patients, were unchanged in 26 patients and a single patient reported worse symptoms. Improvement in these three measures reached statistical significance. Median Quality of Life scores (SF-36) were stable through 12 months with respect to overall mental health (48 [range 32–55] to 48 [range 39–58]) and physical health (63 [range 36–70] to 64 [range 46–73]).

Concordantly high satisfaction rates were demonstrated with procedure outcomes after one or two treatments. Investigator and patient satisfaction with overall breast deformity at 12 months were reported in 58 out of 67 and 45 out of 67 patients respectively. Investigator and patient satisfaction with the overall treatment results through 12 months were reported in 57 out of 67 and 50 out of 67 patients respectively Figs. 1–3.

Resource utilization

The procedure was performed both as an outpatient and inpatient procedure depending on investigator preference,

Table 2
Details of lipoaspirate and ADRC processing.

	Index procedure		6-month treatment	
	Volume (mL)	Time (min.)	Volume (mL)	Time (min.)
	Median (Range)			
Total lipoaspirate harvested	364 (223–570)	32 (15–132)	351 (209–530)	38 (18–106)
Lipoaspirate for ADRC isolation	190 (120–285)	135 (100–210)[a]	171 (109–265)	143 (110–180)[a]
Lipoaspirate for adipose tissue graft	172 (100–290)		174 (100–265)	
Volume of ADRC-enriched graft injected as prepared	140 (35–250)	25 (9–250)	125 (34–220)	30 (10–55)
Estimated volume of "dry" ADRC-enriched graft[b]	91 (23–163)		81 (22–143)	

[a] Total processing time to prepare ADRC-enriched graft.
[b] Dry graft volume is calculated as 65% of wet ADRC-enriched fat graft volume.

patient preference and availability of surgical facilities. Inpatient treatment was performed in 40 of 67 cases at baseline, and in 19 of 24 cases at the optional six month treatment. Hospital length of stay averaged between 7 h and 2 days depending on outpatient or inpatient status.

MRI results

All 68 breasts included in the analysis had defects based on visual assessment, however only 51 defects were detectable on baseline MRI images. Based on this discrepancy, the MRI Assessment Protocol was modified as previously described. Using a five-point Likert scale, changes in breast contour demonstrated an improved or much improved result at six and 12 months compared to baseline in 51 out of 66 patients and 54 out of 65 patients respectively.

Procedural safety

Five serious adverse events (SAEs) occurred over the course of the trial; however no SAEs were related to the ADRC-enriched fat graft injection procedure. Two events, subcutaneous bleeding following liposuction and pelvic bone metastasis, were felt to be unrelated to treatment. The bleeding was attributed to excessive post-operative anticoagulation therapy and resolved without sequelae, while the bone metastasis was considered natural progression of the disease. Injection site cysts were reported as adverse events in 10 patients (14.9%), which is consistent with rates reported in the literature.[18]

In order to elucidate the natural history of grafted fat in these patients, a post-hoc analysis of all RESTORE-2 MRIs was performed. Independent radiographic core laboratory review of six and 12 month MRIs observed new subclinical cysts in 36 patients at 6 months and 46 patients at 12 months, a prevalence rate of 1.1 and 1.7 per patient respectively. Importantly, these cysts were small in nature, 89% $<$ 2 cc at 6 months and 95% $<$ 2 cc at 12 months, suggesting they are self-limiting and their size decreases over time. All cysts were considered benign by radiographic core lab radiologists and no treatment was warranted.

Oncological safety

This trial included patients who had a low risk of breast cancer recurrence.[19] During the course of the trial and 12 month follow-up period, there were no local cancer recurrences. It should be noted that the two patients that were lost to follow-up underwent MRI examination at both six and 12 months. Although these two patients were not

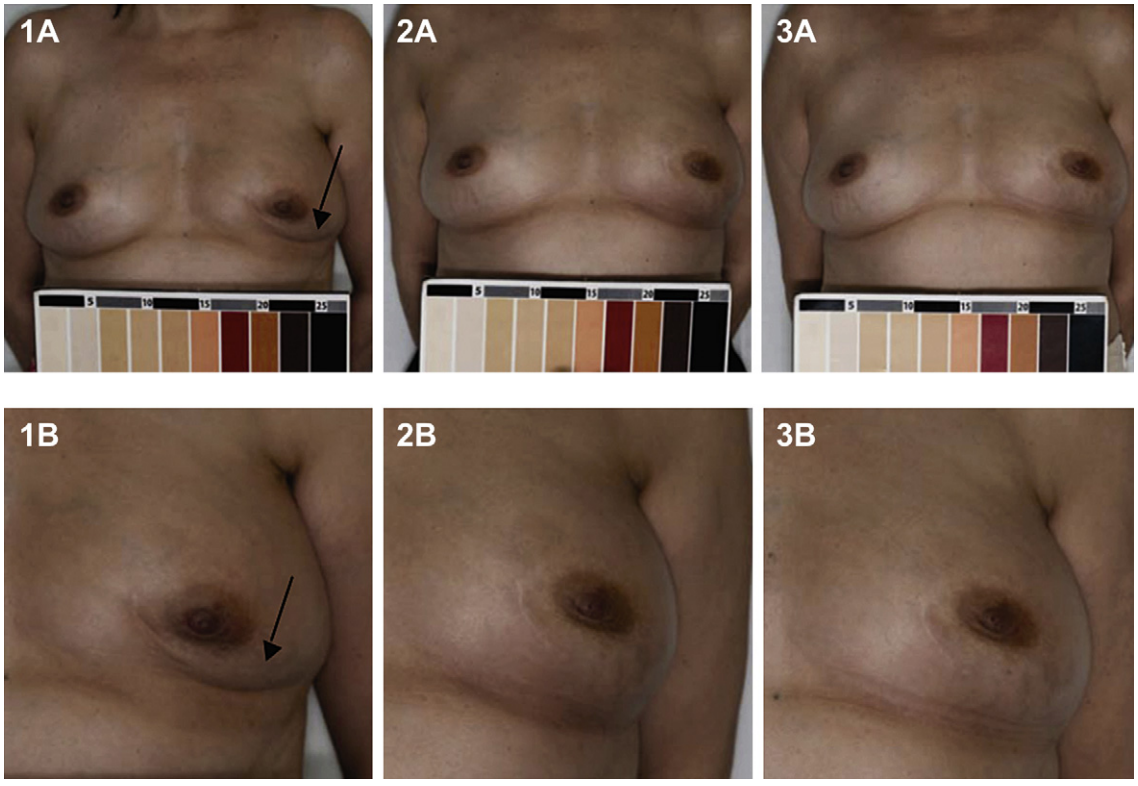

Figure 1. Pre-operative views (1A, 1B) and post-operative views at six (2A, 2B) and 12 months (3A, 3B). This patient presented 28 months following BCT. At time of excision, tumor size measured 0.8 cm in width. Radiation history included 30 treatments with a cumulative dose of 60 Gy. Visual defect estimate at baseline was 100 mL. A total of 385 mL of lipoaspirate was harvested from the abdomen and 150 mL of "wet" ADRC-enriched fat was injected into the left lower pole defect in a single session with a transplantation time of 25 min. At 12 months patient and investigator satisfaction with overall treatment results were reported as "satisfied."

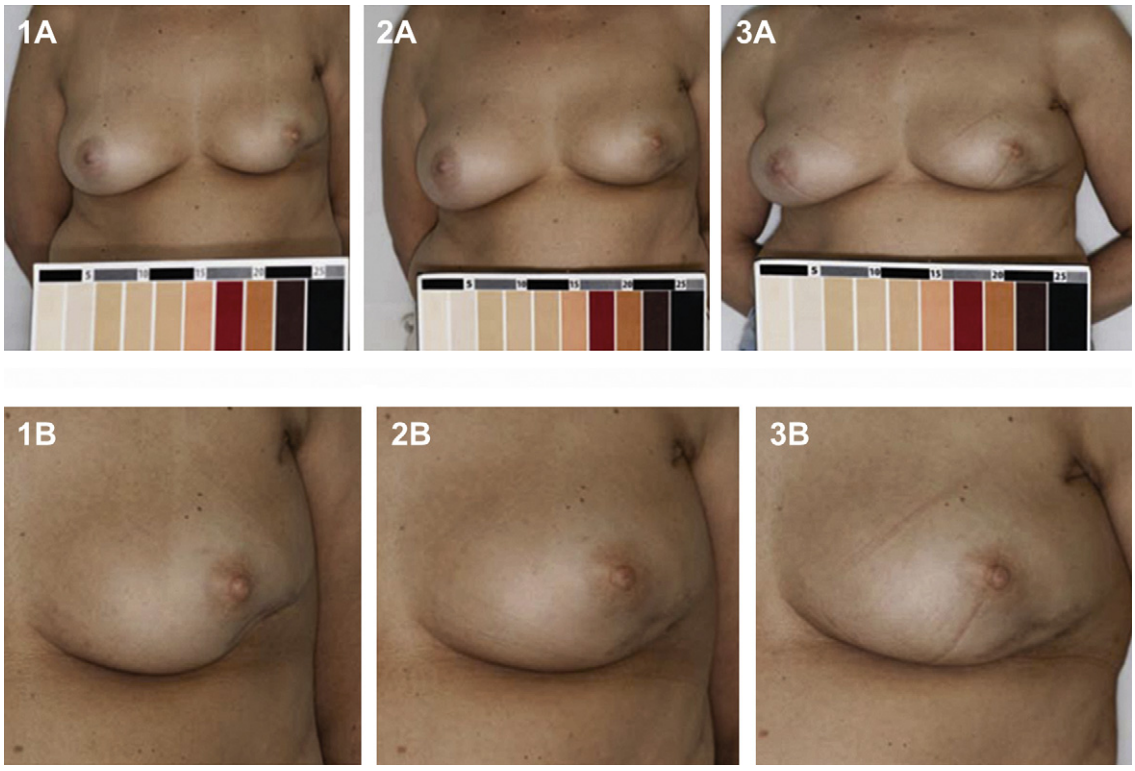

Figure 2. Pre-operative views (1A, 1B) and post-operative views at six (2A, 2B) and 12 months (3A, 3B). This patient presented 41 months following BCT. At time of excision, tumor size measured 2.0 cm in width. Radiation history included 30 treatments with a cumulative dose of 60 Gy. Visual defect estimate at baseline was 100 mL. A total of 420 mL of lipoaspirate was harvested from the abdomen and 150 mL of "wet" ADRC-enriched fat was injected into the left lower-lateral defect in a single session with a transplantation time of 22 min. At 12 months patient and investigator satisfaction with overall treatment results were reported as "satisfied".

evaluated by the investigator, no evidence of local recurrence was present in these two or any of the 67 evaluable patients.

Discussion

This study is the first prospective trial using autologous ADRC-enriched fat grafting for breast reconstruction post-BCT, where all study assessments were pre-specified and all patients treated were included in the assessment of outcomes.[20] RESTORE-2 demonstrated substantial improvement in breast defects after one or two treatments and the results were stable through 12 months. This suggests that volume augmentation at 6 months can be considered permanent barring significant changes in the patient's weight. Additionally, patients and investigators reported continued improvement over time in the tissue quality in or near the defect (i.e., increased skin elasticity, reduction in scar tethering, and reduction in localized edema).

Traditional fat grafting vs. ADRC-enriched fat grafting

Traditional fat grafting in the context of BCT is associated with unpredictable results due to the variability in both the graft (variable methods of fat harvest, processing and delivery), and the site of implantation (extent of scarring, vascularity of the recipient bed, etc.). Multiple procedures are usually required to achieve a satisfactory outcome because much of the graft volume is often absorbed within a few months. Pre-operative planning, appropriate patient selection and standardization of procedural steps are important in reducing this variability. While ADRC-enriched fat grafting successfully treated this group of post-BCT patients, future studies should compare ADRC-enriched fat grafting to other fat grafting techniques in various clinical circumstances. Current clinical data suggests that the addition of ADRCs to a graft may prove most critical when transplanting into a "hostile" recipient bed such as irradiated or scarred tissue.[12]

Limitations

A limitation of this trial was the inability to accurately measure breast volume with MRI. 3-dimensional imaging may have provided a better estimate of volume, but the clinical trial sites did not have access to this imaging modality at the time of the trial. Additionally, most 3-D systems have not been validated for soft tissue volume measurement. Future trials should include validated methods of volume assessment.

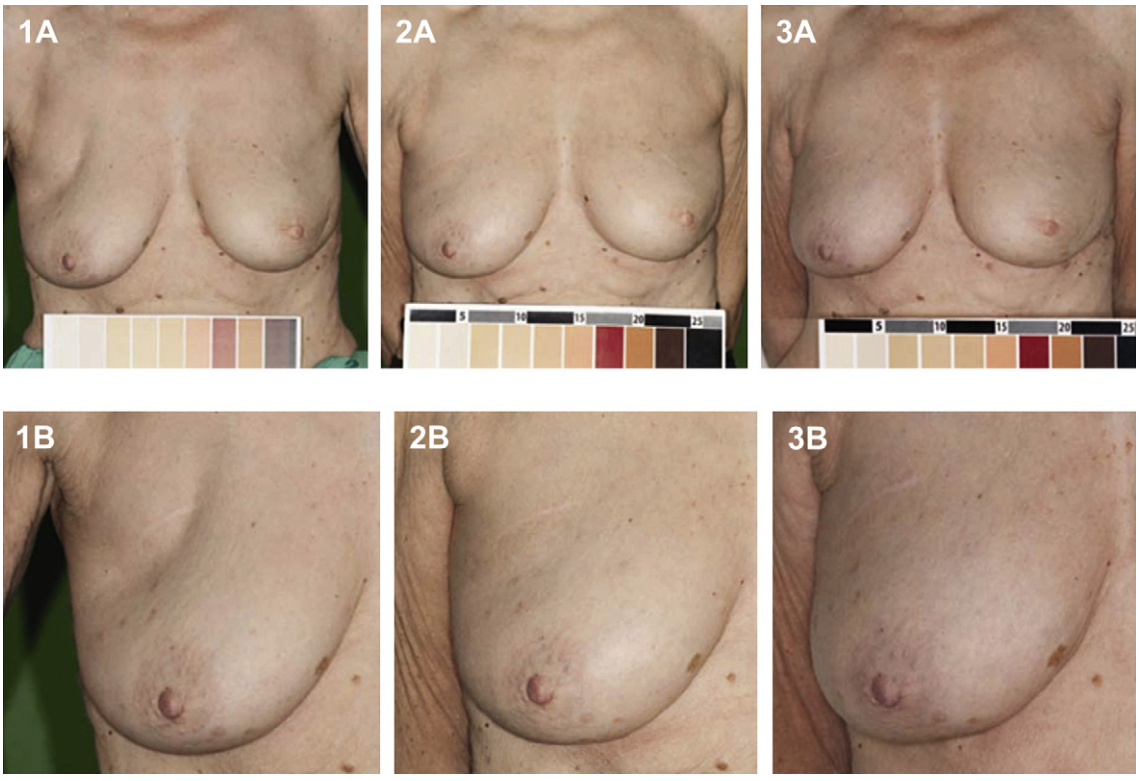

Figure 3. Pre-operative views (1A, 1B) and post-operative views at six (2A, 2B) and 12 months (3A, 3B). This patient presented 136 months following excision of a 3.0 cm benign lesion which did not require radiation. Visual defect estimate at baseline was 150 mL. The initial procedure harvested a total of 560 mL of lipoaspirate from the lateral thighs and 150 mL of "wet" ADRC-enriched fat, which was injected into the right upper-lateral defect with a transplantation time of 30 min. A second procedure was performed at six months with 368 mL of lipoaspirate harvested from the inner thighs and 100 mL of "wet" ADRC-enriched fat was injected into the right upper-lateral defect with a transplantation time of 25 min. At 12 months patient and investigator satisfaction with overall treatment results were reported as "extremely satisfied".

Oncologic safety

This study revealed no local cancer recurrences at 1-year post-index treatment. The current duration of follow-up of this study is inadequate to allow meaningful comment on long-term risk of recurrence. While systematic analysis of cancer recurrence has not been incorporated in most reports, published studies that include more than 2000 patients with several years of follow-up show no evidence of an increased risk of recurrence following fat grafting.[21–25] In one report by Petit et al showing a small apparent increase in recurrence rate in patients with intraepithelial cancer, the authors noted that this difference arose from the unusually low rate of recurrence in the matched control group rather than an unusually high rate in the group receiving fat grafts.[25] The authors noted that this could reflect a sample error. Nonetheless, continued follow-up of all patients treated for breast cancer irrespective of the type of reconstructive procedure applied is indicated.

Conclusion

This study is the first prospective, multi-center clinical trial which evaluates the use of ADRC-enriched fat grafts to treat breast defects post-BCT with or without radiation. Patient and investigator satisfaction with overall breast deformity demonstrated improvement at six months, which continued through to 12 months. Assessment of MRI images conducted by an independent core lab blinded to MRI sequence reported improvement in breast contour in 54 out of 65 patients at 12 months compared to baseline. In summary, RESTORE-2 has demonstrated the safety and efficacy of ADRC-enriched fat grafting in the treatment of soft tissue defects post-BCT and thus, this procedure should be considered as an alternative reconstructive option for women with post-BCT defects. Future comparative studies are needed to determine the incremental benefit of ADRC-enriched fat grafting as compared to traditional fat grafting in various clinical circumstances.

Conflicts of interest

Alex Milstein, MD is an employee of Cytori Therapeutics.

Role of funding source

Cytori Therapeutics, Inc. provided funding along with technical and editorial support for this trial.

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