Efficacy of a Novel Injection Lipolysis to Induce Targeted Adipocyte Apoptosis: A Randomized, Phase IIa Study of CBL-514 Injection on Abdominal Subcutaneous Fat Reduction

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Abstract

Background: CBL-514 is a novel injectable drug that may be safe and efficacious for localized abdominal subcutaneous fat reduction.

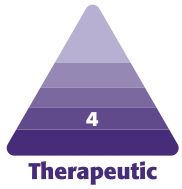
Objectives: The aim of this study was to assess the safety and efficacy of CBL-514 in reducing abdominal subcutaneous fat volume and thickness.

Methods: This Phase IIa, open-label, random allocation study consisted of a 6-week treatment period and follow-up at 4 and 8 weeks following the last treatment. Participants were randomly allocated to receive 1.2 mg/cm^2 (180 mg), 1.6 mg/cm^2 (240 mg), or 2.0 mg/cm^2 (300 mg) of CBL-514 with up to 4 treatments, each comprising 60 injections into the abdominal adipose layer. Changes in abdominal subcutaneous fat were assessed by ultrasound at follow-up visits. Treatment-emergent adverse events were recorded.

Results: Higher doses of CBL-514 (unit dose, 2.0 and 1.6 mg/cm^2) significantly improved the absolute and percentage reduction in abdominal fat volume ($P < 0.00001$) and thickness ($P < 0.0001$) compared with baseline. Although the COVID-19 pandemic halted some participant recruitment and follow-ups, analysis was unaffected, even after sample size limitations.

Conclusions: CBL-514 injection at multiple doses up to 300 mg with a unit dose of 2.0 mg/cm^2 is safe, well-tolerated, and reduced abdominal fat volume and thickness by inducing adipocyte apoptosis. Although other procedures exist to treat abdominal fat, they have limitations and may cause complications. At a dose of 2.0 mg/cm^2, CBL-514 safely and significantly reduced abdominal fat volume by 24.96%, making it a promising new treatment for routine, nonsurgical abdominal fat reduction in dermatologic clinics.

Level of Evidence: 4



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Fat reduction procedures are popular because patients desire aesthetically pleasing body shapes, possibly augmented by pressure exerted by societal and cultural ideals. Localized adiposity is often resistant to diet and exercise and may require interventions such as liposuction.[1] However, liposuction is operator-dependent, costly, suboptimal for nonobese patients, or unappealing for focal adiposity due to its invasiveness.[2] Furthermore, many patients may decline or be unsuitable for surgery.[3] Liposuction also risks long recoveries, scars, hematomas,[4,5] and complications such as bowel perforation[6] or long-term and/or potentially fatal deep vein thrombosis and pulmonary embolism.[4,5]

Noninvasive devices were consequently developed and include cryolipolysis, high-intensity focused ultrasound (HIFU), lasers (eg, low-level laser therapy [LLLT]), radiofrequency (RF), and high-intensity focused electromagnetic field (HIFEM). These treatments have fewer side effects, quicker recoveries, and can address difficult areas such as the submentum[7] and abdomen.[3,8] However, these devices are costly to purchase and operate,[9] slow to deliver results, and require dedicated spaces in clinics. Moreover, their efficacy depends on technical factors including the device's applicator, which risks unevenness if used suboptimally. Devices also have questionable safety records:[10] HIFU can produce nonselective cell necrosis, ecchymosis, and dysesthesia,[1,11] whereas RF[12] may result in transient erythema, burns, and bruises.[13] Longer 1060-nm laser treatments[14] can produce dermal injury and subcutaneous nodules. Cryolipolysis may cause dysesthesia, erythema, and edema,[15] severe frostbite,[16] and asymmetry.[17] Up to 0.47% of patients have developed postcryolipolysis paradoxical adipose hyperplasia.[18]

Injection lipolysis is a minimally invasive, targeted, fat reduction alternative that can be customized to the patient's level and area of fat accumulation.[19] One example of this uses deoxycholic acid (DCA), an adipocytolytic component of human bile acid. Although DCA mainly affects fat cells, it is relatively nonselective and has some ability to lyse muscle and other adjacent nonfatty tissues. DCA treatment is associated with transient side effects such as injection site pain, erythema, ecchymosis, and numbness.[20,21] Despite injection lipolysis being used for decades[22] in nonsurgical, localized fat reduction,[23] only Kybella (ATX-101; Allergan Aesthetics, Irvine, CA) which contains DCA, is approved in the United States, and only for submental fat reduction.[19,24,25] In Phase II and III studies of ATX-101,[26-28] dose-dependent, durable, and targeted adipolysis occurred from 1 day postinjection, leading to a significant reduction in submental fat.[20] However, skin necrosis, ulceration, marginal mandibular nerve neuropraxia, and infections are known side effects of ATX-101, which is not indicated for fat reduction in other body areas.[29,30] No injectable drug currently offers both efficacy and safety for localized subcutaneous fat reduction in larger body areas.

CBL-514 (Caliway Biopharmaceuticals, New Taipei City, Taiwan) is an injection lipolysis drug. CBL-514 induced adipocyte apoptosis and lipolysis in vitro and promoted adipose cell apoptosis and reduced subcutaneous adiposity in targeted areas in animal studies. Our nonclinical studies showed that CBL-514 inhibits the cell survival kinase DYRK1b; upregulates the apoptosis mediators caspase 3 and Bax/Bcl-2; and induces dose-dependent adipocyte apoptosis in vivo and in vitro (Caliway Biopharmaceuticals, unpublished confidential reports).[31] To evaluate its safety and efficacy in humans, we conducted a Phase IIa investigation of CBL-514 at various doses for abdominal subcutaneous fat reduction.

METHODS

Study Design and Treatment

This Phase IIa (ClinicalTrials.gov NCT 04698642), proof-of-concept, open-label, random allocation study was conducted at a single site in Australia between February and November 2020. CBL-514 was administered over up to 4 treatments at 2-week intervals, in up to 60 injections at 1.58-cm intervals per injection, into the abdominal subcutaneous adipose layer. Treatment covered a 150-cm^2 area and included 2 follow-up visits at 4 and 8 weeks after the last treatment. Participants were randomly allocated to receive either 1.2 mg/cm^2 (0.6 mL/injection; 180 mg/treatment), 1.6 mg/cm^2 (0.8 mL/injection; 240 mg/treatment), or 2.0 mg/cm^2 (1 mL/injection; 300 mg/treatment) of CBL-514. The efficacy of CBL-514 at changing abdominal subcutaneous fat was assessed by ultrasound imaging at 2 follow-up visits and compared to baseline values. Safety assessments included the incidence and severities of treatment-emergent and serious adverse events (TEAEs and serious AEs). Changes in clinical laboratory, vital signs, and electrocardiographic parameters were also assessed. The study protocol was reviewed and approved by Human Research Ethics Committee, Bellberry Limited, Eastwood, South Australia, Australia (approval number 2017-12-972-AB).

Inclusion and Exclusion Criteria

Enrolled participants were between 18 and 64 years of age and included women who were not pregnant or lactating, and men. Participants were placed on medical birth control if they or their partners had childbearing potential. At baseline (Day 1), all participants had a BMI >18.5 and <35 kg/m^2, a waistline circumference (WC) of 80.0 to 110.0 cm, and a treatment site skinfold thickness of ≥30.0 and ≤60.0 mm as measured by skinfold caliper. Participants had to maintain a stable weight and lifestyle for at least 3 months before screening. Participants were discontinued if their body

weight increased or decreased by ≥5% of baseline values, or if they received weight reduction treatments within 3 months of the study, liposuction within 12 months of the study, or any nonsurgical fat reduction procedure on the treatment area within 6 months of the study. All participants provided their written informed consent before enrollment. The trial was conducted according to the provisions of the Declaration of Helsinki, International Conference on Harmonisation Good Clinical Practice guidelines, and applicable local regulatory requirements. A Human Research Ethics Committee approved the study protocol and its amendments.

Participant Withdrawal

Due to the COVID-19 pandemic, the study recruited 43 of the originally planned 47 participants, with 65.1% (n = 28) of enrolled participants completing all study visits and 15 participants withdrawing from the study. Of these 15 participants, 8 withdrew due to adverse events, including injection site pain (n = 3), injection site pruritus (n = 1), injection site swelling (n = 1), repeated urticaria (n = 1), body rash (n = 1), and fever (n = 1). The body rash reported was not study drug related. Reasons for early termination of the remaining 7 participants included withdrawn consent, meeting the criteria for withdrawal due to body weight control, and loss to follow-up.

Randomization

Participants were originally planned for fixed, blocked randomization into 3 dose groups in a 1:1:1 ratio. Due to COVID-19 pandemic restrictions, recruitment of new participants was halted and restarted only after lockdowns were lifted. To minimize impacts to the study timeline and the possibility of treatment ineffectiveness, the recruitment of low-dose group participants was permanently suspended when 7 participants were enrolled. Medium- and high-dose group participants continued enrollment so that planned randomization of a total of 40 participants into 2 dose groups, in a 1:1 ratio, could be completed. Due to recurrent COVID-19 outbreaks, the study permanently suspended recruitment when 36 participants were eventually enrolled in the medium- and high-dose groups in addition to 7 participants previously enrolled in the low-dose group.

Efficacy Endpoint Measures

The primary endpoints were the ultrasound-measured reduction and percentage of change in abdominal subcutaneous fat volume between baseline and follow-up visits. The secondary endpoints were the ultrasound-measured reduction in abdominal subcutaneous fat thickness between baseline and follow-up visits.

To ensure accuracy when measuring subcutaneous adipose tissue (SAT) thickness with ultrasound, the sonographer measured 5 points on the abdomen (Supplemental Figure 1). A central point situated 3 cm below the umbilicus was denoted as the center of the treatment area. A second and third point were placed 7 cm to the left and to the right of the central point, respectively. A fourth and fifth point were located at each participant's right and left tendinous intersections, which were determined and mapped out by the sonographer when they performed their initial scans. Three ultrasound scans were conducted per point to obtain an average value. To avoid fat compression artifacts, no probe pressure was applied. Measurements were all taken longitudinally and aligned with the deeper muscle fibers, and only the SAT layer was measured. A uniform, tendinous intersection was used as a deep landmark to facilitate consistent and reproducible measurements. This landmark was identified for each participant on the first day (Day 1) predose. To minimize variations from different personnel operating the ultrasound, 3 specific technicians were identified by the imaging center. These individuals were the only staff designated to conduct and collect study measurements. The SAT thickness was the average thickness of 5 points scanned by ultrasound. To calculate the loss in abdominal fat volume, the cuboid volume formula was applied:

$$\text{volume} = \text{basal area} \times \text{height}$$

The basal area was a defined treatment area of 150 cm^2. The height was the average SAT thickness measured at 5 points scanned by ultrasound. Ultrasound imaging of the SAT was performed only to obtain thickness measurements. No other assessment or observation was performed.

Safety Endpoint Measures

Safety assessments included TEAE incidence and severity, laboratory assessments, vital signs, electrocardiography (ECG), and physical examinations. TEAEs were summarized by System Organ Class, preferred term, severity, and suspected relationship to study treatment. Treatment-related TEAE was defined as any TEAE reported as being "possible," "probable," or "definite" in relation to them being due to the study treatment. Laboratory assessments included biochemistry, hematology, coagulation, and urine dipstick. As part of the safety endpoint, clinical laboratory tests, ECG, and vital signs were assessed vs baseline to monitor the overall health condition during the study.

Photography

Photographs were taken of the participants' frontal and profile views of their abdomens at all visits to record treatment site responses and/or reactions.

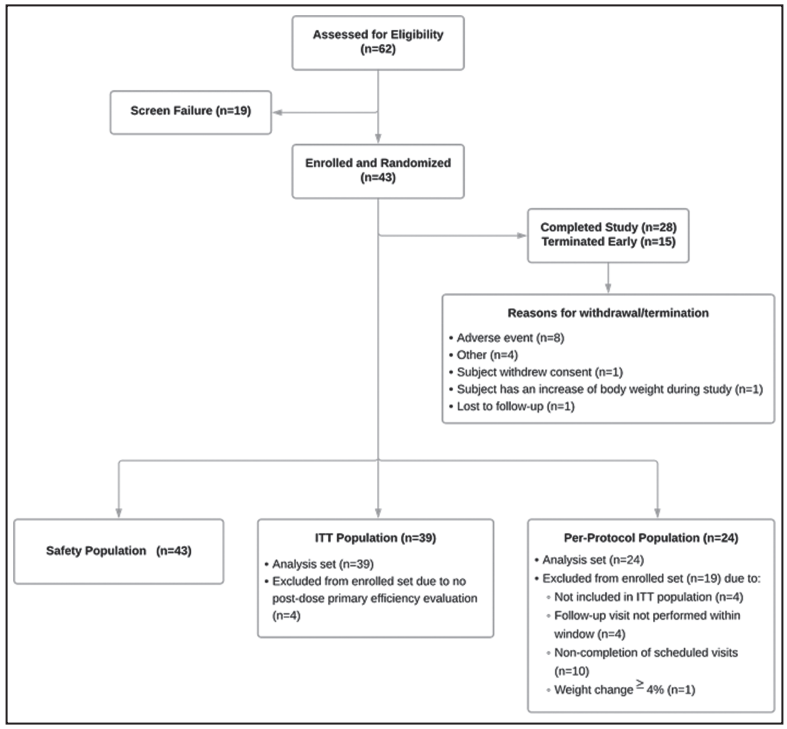


Figure 1. Participant disposition.

Statistical Analysis

Forty-seven participants were planned for enrollment. Because this study phase was exploratory, no previous formal sample size hypothesis testing was performed, and therefore no changes to the planned analysis were needed.

The efficacy endpoint was assessed in the eligible intent-to-treat (ITT) cohort that had baseline evaluations and at least 1 postbaseline primary efficacy assessment. The efficacy analysis was repeated on the per-protocol (PP) participants who completed all dosing schedules and 2 follow-up visits, with no major schedule or body weight deviations, and no major efficacy-related protocol violations. The efficacy endpoints—the reduction of abdominal subcutaneous fat volume and fat thickness—were summarized by descriptive statistics including 95% CIs for the mean and corresponding *P* values. All efficacy analyses (unless specified) were performed at a 2-sided 5% level of significance.

The safety endpoint was evaluated in all enrolled participants receiving at least 1 dose of the study drug and TEAEs were summarized. Changes in clinical laboratory tests and vital signs from baseline were summarized by descriptive statistics. Physical examination findings, injection site assessments, and ECGs were summarized descriptively.

RESULTS

Participants

A total of 43 participants (mean age, 49.2 years; range, 25.0-64.0 years; 27 females [69.2%], 12 males [30.8%]) were enrolled and randomly allocated to 3 designated dose levels of CBL-514 (1.2, 1.6, and 2.0 mg/cm^2). Of the 43 enrolled participants, 7, 18, and 18 participants received CBL-514 at doses of 1.2, 1.6, and 2.0 mg/cm^2, respectively. Of those enrolled, 90.7% (n = 39) were included in the ITT population and 55.8% (n = 24) were included in the PP population (Figure 1). Participants who completed at least 1 follow-up visit postdose had an average of 51.6 days (range, 7-106 days) of follow-up from the last treatment visit. Participant demographics (Table 1) were similar across the different dose groups. The mean change in weight from baseline to the end of study (EOS) visit was 1.1 kg, and the mean change of BMI from baseline to EOS visit was 0.3. Across dose groups, none of the anthropometric changes occurring during the study were meaningful (Table 1).

Ultrasound Measure for SAT Thickness

The sonographers followed the specified ultrasound measurement manual directions to ensure that ultrasound scan points were consistently relocated in the same areas of each participant's abdomen. Any errors between 3 repeated ultrasound measurements per scan were maintained within a reasonable range of approximately 1 mm. The point at 7 cm to the right of the central point of ultrasound images from a representative participant is shown in Figure 2.

Primary Efficacy Outcomes

The study met the primary efficacy endpoints of reductions in volume and percentage change of abdominal

Table 1. ITT Participant Demographics and Changes in Baseline Characteristics.

Variable	CBL-514 1.2 mg/cm^2, N = 7	CBL-514 1.6 mg/cm^2, N = 16	CBL-514 2.0 mg/cm^2, N = 16	Overall N = 39
Demographics				
Age (years), median [SD]	49.0 [13.2]	54.0 [9.7]	48.0 [9.4]	50.0 [10.2]
Male, n (%)	1 (14.3)	6 (37.5)	5 (31.3)	12 (30.8)
Female, n (%)	6 (85.7)	10 (62.5)	11 (68.8)	27 (69.2)
Race, n (%)				
Asian	1 (14.3)	0	0	1 (2.6)
White	6 (85.7)	16 (100.0)	15 (93.8)	37 (94.9)
Other	0	0	1 (6.3)	1 (2.6)
Change in characteristics				
Weight (kg), mean [SD]				
Baseline	70.0 [9.7]	73.4 [11.4]	76.1 [13.0]	73.9 [11.7]
First follow-up visit	69.6 [8.9]	74.0 [12.1]	75.3 [13.4]	73.7 [12.0]
Second follow-up visit (EOS)	66.5 [6.4]	75.1 [12.2]	77.9 [13.5]	75.0 [12.4]
BMI (kg/m^2), mean [SD]				
Baseline	25.0 [3.6]	26.4 [3.5]	26.3 [3.0]	26.1 [3.3]
First follow-up visit	24.9 [3.3]	26.7 [3.8]	26.3 [3.1]	26.2 [3.4]
Second follow-up visit (EOS)	24.2 [2.1]	26.8 [3.8]	26.8 [3.0]	26.4 [3.3]

EOS, end of study; ITT, intention-to-treat; n, number of participants in each count; N, number of participants in the ITT population in each treatment group; SD, standard deviation.

subcutaneous fat over the treated area vs the baseline. In addition, all CBL-514 doses (1.2, 1.6, and 2.0 mg/cm^2) reduced the absolute and percentage volumes of fat from baseline (Figure 3), with increasing doses of CBL-514 leading to a correspondingly greater reduction in fat volume.

Statistically significant differences were observed for the least-square mean change of abdominal subcutaneous fat volume in absolute and percentage change from baseline at both follow-up visits in the ITT population, at doses of 1.6 and 2.0 mg/cm^2 ($P < 0.001$ and $P < 0.00001$, respectively). At the first and second follow-up visits, CBL-514 1.2 mg/cm^2 reduced fat by 5.38% and 4.16%, respectively, versus 16.53% and 13.88% with CBL-514 at 1.6 mg/cm^2, respectively, and 23.13% and 21.85% with CBL-514 at 2.0 mg/cm^2, respectively (Figure 3). Fat reduction was statistically significant between CBL-514 1.6 mg/cm^2 and 2.0 mg/cm^2 at both follow-up visits.

In the PP population, the least-square mean reduction in fat volume from baseline at CBL-514 2.0 mg/cm^2 was –119.82 mL (–24.96%) at the first follow-up and –104.82 mL (23.54%) at the second follow-up. The PP population had greater absolute and percentage changes in fat volume from baseline than the ITT population, indicating that compliance with visit schedules likely contributed to fat reduction.

The study had no control group. However, when CBL-514 1.2 mg/cm^2 was used as a hypothetical control group, a significant difference in the absolute and percentage change in fat volumes from baseline was observed for CBL-514 2.0 mg/cm^2 vs CBL-514 1.2 mg/cm^2 at the first ($P < 0.01$ and $P < 0.001$, respectively) and second follow-ups ($P < 0.05$ and $P < 0.01$, respectively).

Secondary Efficacy Outcomes

Secondary efficacy endpoints were met. At both follow-up visits, CBL-514 1.6 and 2.0 mg/cm^2 significantly reduced abdominal subcutaneous fat thickness vs baseline ($P < 0.001$ and $P < 0.00001$, respectively), with increasing doses increasing these reductions. At 2.0 mg/cm^2, the least-square mean change of fat thickness from baseline was –7.39 mm at the first follow-up and –6.54 mm at the second follow-up (both $P < 0.00001$). Changes from baseline were greater in the PP population than the ITT population. In the PP population with CBL-514 2.0 mg/cm^2, the change of ultrasound-measured fat thickness from baseline was –7.66 mm at the first follow-up and –7.16 mm at the second follow-up ($P < 0.00001$ and $P = 0.00001$, respectively). When CBL-514 1.2 mg/cm^2 was used as the hypothetical control, fat thickness was significantly reduced from baseline in the 2.0-mg/cm^2 group at the first follow-up ($P < 0.05$).

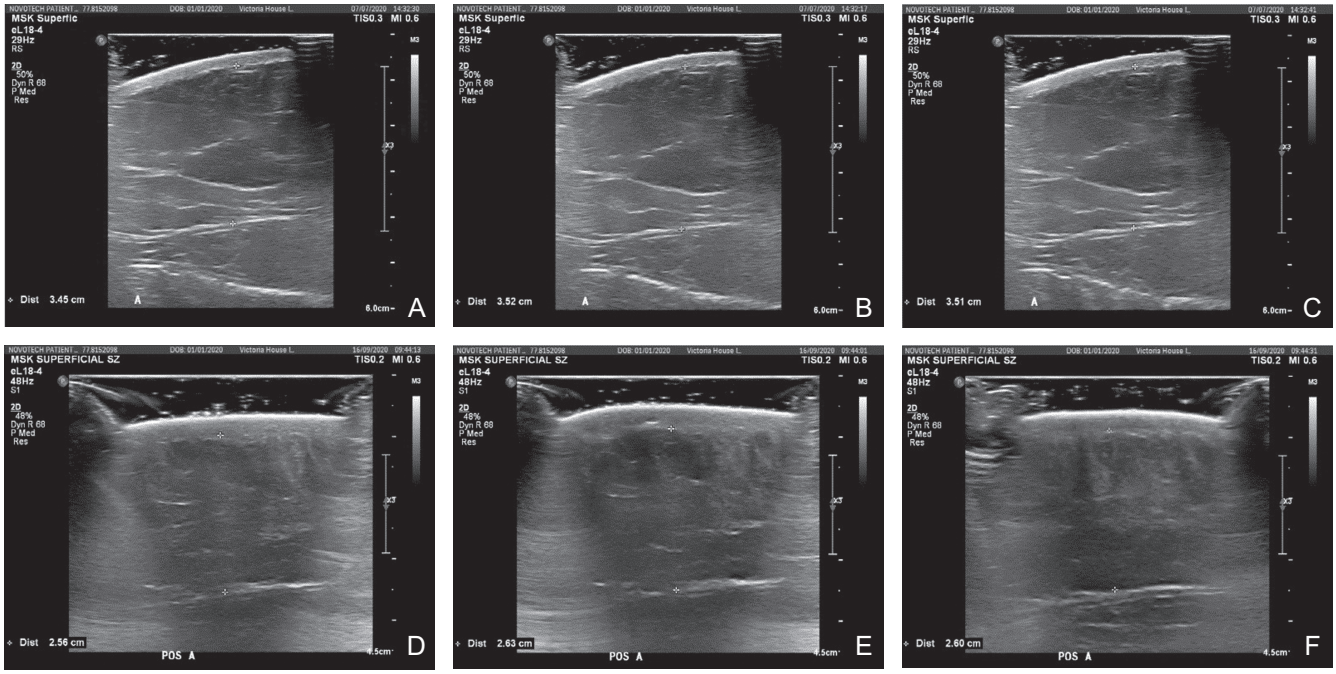


Figure 2. Ultrasound images of subcutaneous fat thickness at (A) baseline 1; (B) baseline 2; (C) baseline 3; (D) first follow-up 1, 4 weeks after last treatment; (E) first follow-up 2, 4 weeks after last treatment; and (F) first follow-up 3, 4 weeks after last treatment.

Safety Outcomes

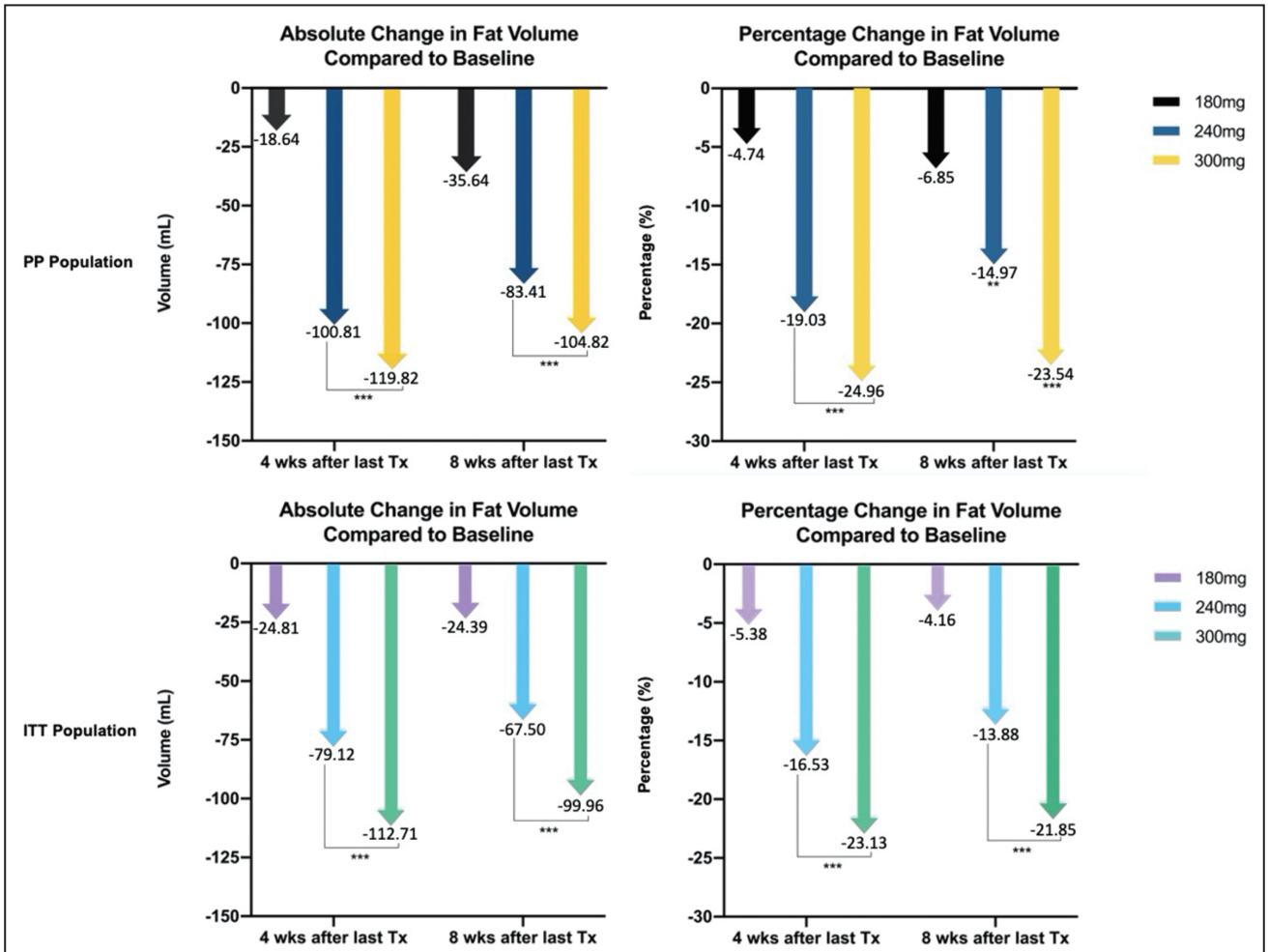
All participants reported at least 1 TEAE, the majority of which resolved completely within 2 weeks. Injection site–related TEAEs were most common, including bruising, erythema, pain, swelling, pruritus, and induration. TEAEs were mild (80.13%) or moderate (19.06%). Nine TEAEs were severe, including 8 injection site pain events in 4 participants (across all doses) and 1 whole-body rash (with 2.0 mg/cm^2) at 8 days posttreatment which the investigator considered unrelated to CBL-514. Seven participants withdrew from the study due to treatment-related TEAEs, which were mostly injection site pain. No serious AEs occurred, and no TEAEs were life-threatening or resulted in death. The majority of the TEAEs were either recovered or recovering at the end of the study visit.

Four participants had mild, clinically significant, and abnormal laboratory results, which were possibly related to CBL-514: 2 participants had both hypertriglyceridemia and hypercholesterolemia, and 2 participants had only hypercholesterolemia (Supplemental Table 1 shows their triglyceride and total cholesterol values). These participants had higher baseline values close to or above the upper limit of the normal triglyceride and cholesterol range. The time to onset of clinically significant abnormalities in triglyceride or total cholesterol levels varied from participant to participant. Considering the time to onset, the investigator could not determine if the elevated blood lipids were entirely related to the study drug. Except for the aforementioned reports of triglyceride and total cholesterol levels, no other CBL-514–related and clinically significant laboratory results, or abnormalities in vital signs and ECG parameters, were observed.

To mitigate injection site pain, topical anesthetic cream was applied to the treatment area and ice compression was used before and after study drug injections in all participants. Analgesia was also used at the investigator's discretion when participants experienced injection site pain. One notable injection site reaction was postdose pruritus. Although all incidences of this were mild or moderate, the characteristics of pruritus caused a notable inconvenience for the participants' daily life, but its etiology is not well understood. Therefore, antihistamines were recommended to participants if needed to prevent scratching due to injection site pruritus. Injection site pruritus occurred in 95.3% of participants, 20 of whom took antihistamines. Through this management strategy, the incidence and severity of injection site reactions were similar across all dose level groups.

Photography

Digital photographs were captured with a conventional point-and-shoot digital camera for preliminary evaluations of CBL-514 effect on treatment area. Photographs of the target areas before and after CBL-514 treatment for 2 participants are shown at baseline, 4 weeks (first follow-up) and 8 weeks (EOS) after the last treatment as representative of the cohort (Figures 4, 5). Participants' body weight retained stable during the study (Table 2).



P-values of Fat Volume Change

Group	Unit dose (mg/cm^2)	Absolute Change		Percentage Change	
		4 weeks†	8 weeks†	4 weeks†	8 weeks†
ITT Population	1.2	0.29171	0.32228	0.16466	0.35595
	1.6	<0.00001	0.00016	<0.00001	0.00003
	2.0	<0.00001	<0.00001	<0.00001	<0.00001

**: $p < 0.01$
***: $p < 0.001$
Abbreviations: †Follow-up point after last treatment; ITT = intent-to-treat; wks = weeks; Treatment = treatment

Figure 3. Fat volume change.

DISCUSSION

In this Phase IIa, randomized, open-label, parallel, dose-finding study, abdominal subcutaneous fat was significantly reduced with CBL-514 doses of 1.6 and 2.0 mg/cm^2. Notably, at both follow-up visits, the greatest reductions occurred at the highest dose (2.0 mg/cm^2) of CBL-514, with absolute abdominal fat volumes significantly decreasing in the ITT population regardless of dose. Correspondingly, percentage reduction with the 2.0-mg/cm^2 dose was significantly higher than with the 1.6- or 1.2-mg/cm^2 doses, and also statistically significant vs the lowest dose, 1.2 mg/cm^2. At both follow-up points, ultrasound fat thickness was statistically significantly different from baseline for the 1.6-mg/cm^2 ($P < 0.0001$ and $P < 0.001$, respectively) and 2.0-mg/cm^2 ($P < 0.00001$ at both points) doses, with the highest dose showing a statistically significant change vs the lowest dose ($P < 0.05$). Additionally, the PP population had

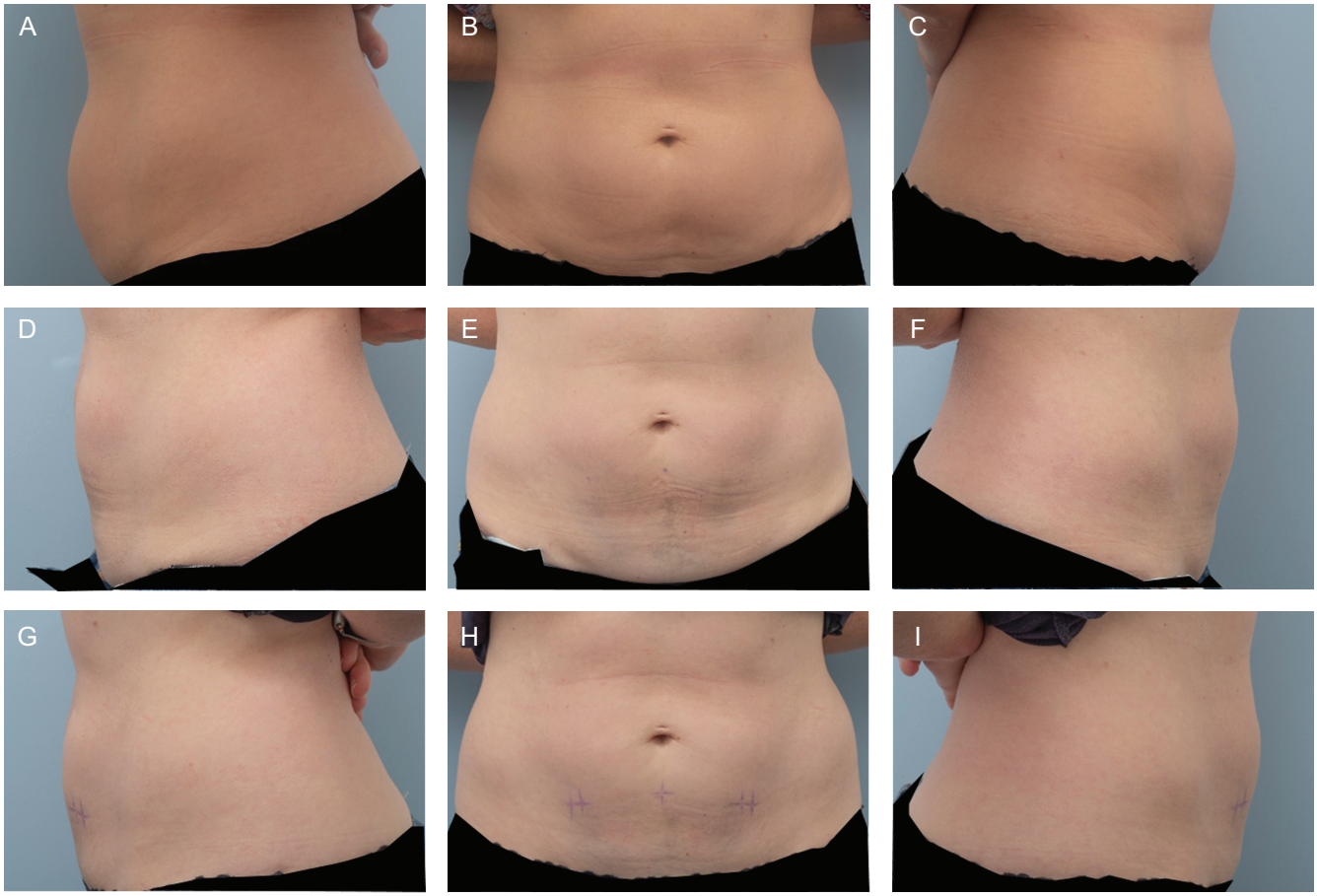


Figure 4. Photographs of the first representative participant, a 50-year-old female, pre- and posttreatment at (A) baseline left view; (B) baseline front view; (C) baseline right view; (D) first follow-up left view, 4 weeks after last treatment; (E) first follow-up front view, 4 weeks after last treatment; (F) first follow-up right view, 4 weeks after last treatment; (G) second follow-up left view, 8 weeks after last treatment; (H) second follow-up front view, 8 weeks after last treatment; and (I) second follow-up right view, 8 weeks after last treatment.

a slightly greater overall reduction in absolute and percentage change of fat volumes at both follow-up visits, underscoring the importance of body weight maintenance and adherence to treatment schedules.

As WC measurements can be confounded by factors such as diet or bloating, we used standardized ultrasound procedures to ensure technical consistency and reliability of data collection when measuring subcutaneous fat thickness and volume. We found ultrasound to be superior to WC and caliper measurements during our trial. Previous studies evaluating WC and fat thickness found moderate reductions with cryolipolysis,[32] HIFEM,[33] LLLT,[34] RF,[35] and HIFU.[36] MRI showed that HIFEM reduced adipose tissue thickness by 18.6% (0.43 cm; $P < 0.0001$) 2 months posttreatment,[37] whereas ultrasound showed that HIFEM reduced abdominal adiposity by 15.7% ($P < 0.05$) at 8 weeks posttreatment.[33] Ultrasound after 1060-nm diode laser treatment showed that abdominal adiposity was reduced by 4.92% at 6 weeks and 8.55% ($P < 0.0001$) at 12 weeks posttreatment.[3] However, ultrasound found no statistically significant reductions after LLLT[38] and, paradoxically, thickness increased in some participants. Using 3-dimensional photography[4] and comparing vs control, a statistically significant absolute fat volume loss of 56.2 mL ($P < 0.0001$) was observed 2 months after cryolipolysis, as well as a mean absolute difference of 39.6 mL between treated and control areas.

In many medical aesthetics clinics, noninvasive fat reduction is now routinely performed with technologies that damage adipocytes, cause them to undergo necrosis,[39] and extrude lipid droplets which become phagocytosed by macrophages. This principle is applied in injectable lipolysis through the use of formulas comprising phosphatidylcholine or DCA plus phosphatidylcholine. However, such formulations are unregulated, their safety is questionable,[21] and the on-label use of ATX-101 is currently approved only for submental fat treatments.[40] In the REFINE-2 trial,[41] MRI showed that ATX-101 reduced submental fat volume from baseline by 8.6% at 12 weeks after last treatment and the reduction in the ATX-101 group was

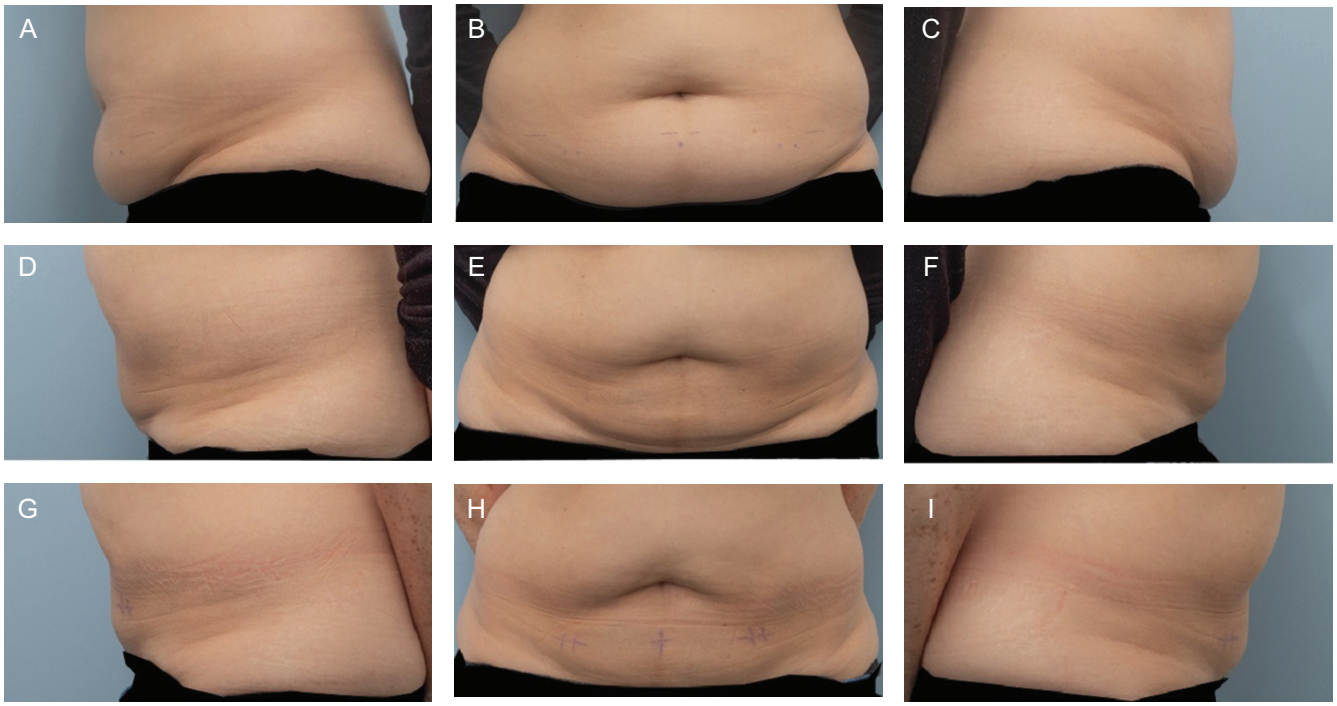


Figure 5. Photographs of the second representative participant, a 46-year-old female, pre- and posttreatment at (A) baseline left view; (B) baseline front view; (C) baseline right view; (D) first follow-up left view, 4 weeks after last treatment; (E) first follow-up front view, 4 weeks after last treatment; (F) first follow-up right view, 4 weeks after last treatment; (G) second follow-up left view, 8 weeks after last treatment; (H) second follow-up front view, 8 weeks after last treatment; and (I) second follow-up right view, 8 weeks after last treatment.

greater compared with the placebo group ($P < 0.001$). Although ATX-101 is associated with mild or transient side effects, 15% of male patients undergoing submental fat reduction developed nonscarring alopecia.[42] Only 1 study indicated resolution or improvement of the condition in 5 out of 8 men; thus, further work is needed to establish if certain patients are predisposed to this event.[43] At 10 mg/mL, ATX-101 damages the marginal mandibular nerve (MMN) myelin sheath; thus, ATX-101 injection also causes the rare but serious complication of MMN paresis.[28,41,44] In the REFINE trials, MMN paresis affected up to 4.3% of patients[45] and although it resolved within 26 days,[46] permanent facial expression disruptions, salivary incontinence, and difficulties in swallowing and drinking can still occur.[44] In contrast to ATX-101, CBL-514 only led to mostly mild or moderate TEAEs which were unaffected by different drug doses, were related to injection site reactions, and resolved before subsequent treatments. Importantly, larger areas such as the abdomen require correspondingly large product quantities and are cost-prohibitive, and although ATX-101 can significantly reduce fat in submental areas, this capacity remains unknown for other body areas. Thus, the unmet need for noninvasive body contouring persists.

Our data imply better efficacy and safety profiles with CBL-514 than with ATX-101 or other noninvasive lipolysis procedures. Nevertheless, as this dose-finding study aimed to determine the most effective dose for future clinical trials, potential study limitations include a relatively small sample size, a lack of placebo controls, and the use of ultrasound alone to evaluate subcutaneous fat. Future studies will need to evaluate placebo controls to justify the efficacy of CBL-514 and compare CBL-514 to current noninvasive lipolysis procedures in standardized, head-to-head trials.[31] Future investigations will also need to establish if there is a positive correlation between ultrasound and other methods of assessing fat reduction efficacy. Before conducting Phase III studies, accurate assessment methods for SAT reduction will need to be identified. Notable injection site reactions—pain and pruritus—occurred in our study. Injection site pain is a common postprocedural event and can be easily managed by analgesia. However, the cause of injection site pruritus remains unknown. Additionally, the elevated blood lipids in 4 participants may indicate the metabolism of lysed fat posttreatment. It is not currently possible to rule out the induction of higher blood lipids due to lipolysis mediated by CBL-514, and a larger sample size is required in future studies to establish causality. Although study drug metabolism and clearance in the human body were not investigated in this study, all test results associated with liver and renal function were normal postdose.

Table 2. Body Weight Change of Participants in Figures 4 and 5.

Variable	Day 1 baseline	First follow-up visit	Second follow-up visit
Body weight (kg; lb)			
Participant 1	57.1; 125.9	58.2; 128.3	57.6; 127.0
Participant 2	67.4; 148.6	68.0; 149.9	68.2; 150.4
BMI (kg/cm^2)			
Participant 1	20.5	20.9	20.7
Participant 2	25.1	25.3	25.4

No biopsies were conducted in this study as this was a dose-finding study to first evaluate the efficacy of CBL-514 and then identify an optimal dosage. Nevertheless, the results of nonclinical studies support our proposed mechanism of subcutaneous fat reduction via CBL-514-induced adipocyte apoptosis (Caliway Biopharmaceuticals, unpublished confidential reports).[31] Specific molecules in the apoptosis pathway serve as biomarkers indicating the induction of apoptosis, such as Caspase-3 activation,[47,48] Annexin V/propidium iodide–positive double-labeling,[49] and increased Bax/Bcl-2 ratio.[50] Our unpublished, internal, nonclinical studies with these biomarkers demonstrated CBL-514 activation of adipocyte apoptosis, leading to lipolysis. Mature 3T3-L1 adipocytes treated with CBL-514 demonstrated significantly higher Caspase-3 activation and Bax/Bcl-2 ratios than control or ATX-101–treated adipocytes. There was also a time-dependent correlation between Bax/Bcl-2 ratio and CBL-514 treatment duration, with a higher ratio when mature 3T3-L1 adipocytes were treated for longer. Annexin V/propidium iodide–positive labeling was significantly higher on CBL-514–treated adipocytes than on control and ATX-101–treated adipocytes. In our internal rodent studies, local subcutaneous fat tissues in the CBL-514 treatment area were reduced significantly and dose-dependently, compared with controls, with Bax/Bcl-2 ratio also significantly increased in the fat of CBL-514 groups compared with controls. In our repeated dose toxicology studies in canines and rodents, no CBL-514–related microscopic finding (or necrosis) was observed in hematoxylin-eosin–stained histology slides of organs and tissues surrounding the injection site. Together, these results suggest that CBL-514 functions by inducing adipocyte cell death by apoptotic mechanism, thus reducing subcutaneous fat in the treatment area. In support of this, the current early Phase IIa study now shows that CBL-514 reduces abdominal subcutaneous fat, which we believe occurs through adipocyte apoptosis and lipolysis. We therefore expect CBL-514 to produce favorable outcomes in subcutaneous fat reduction in other body areas, regardless of size.

With only 1 injection lipolysis formulation currently approved (and only for the submental region), it is important to highlight the nonclinical benefits of injection lipolysis over the more established, fat-reducing devices. Although effective and used routinely, most devices are expensive to initially purchase, with additional hidden costs such as consumables, parts maintenance and repairs, installation and backup, protective equipment, and upgrades or add-ons.[51] Nonfinancial device considerations include their large footprint and long treatment durations (eg, cryolipolysis treatments take 60 minutes per site with most abdomens needing 4 treatments). Injection lipolysis does not require expensive machinery, dedicated space (or power outlets with specific voltages), or device-customized parts and consumables. Moreover, injection lipolysis treatment may be individualized and targeted directly to the patient's particular fat distribution, whereas fat-reducing machine applicators may have more difficulty delivering treatments as evenly or in an individualized manner. CBL-514 may also be used over a much larger body area—as illustrated by abdominal treatment in this study—than other approved injection lipolysis methods. Practitioners in larger practices may also need to transport and share devices, but this is unnecessary with injection lipolysis, making such aesthetics treatments more accessible to a wider and more diverse pool of patients.

Despite the fact that a designated comparative control group was not included, participants on low-dose CBL-514 1.2 mg/cm^2 served as a surrogate control cohort, allowing us to confirm that CBL-514 2.0 mg/cm^2 significantly reduced subcutaneous fat. In addition, to better understand the implementation of CBL-514, future clinical studies will explore the optimal number of injections and treatment sessions needed, and the optimal CBL-514 treatment protocol. Our current study also aimed to find the most effective dose from 1.2 to 2.0 mg/cm^2 and confirm CBL-514 safety rather than to evaluate long-term efficacy, thus even though a favorable outcome was demonstrated over a short duration (8 weeks), further long-term follow-up investigations are needed to confirm the robustness and consistency of fat reduction by CBL-514. Our safety and efficacy results highlight the promise of CBL-514 in selectively inducing adipose apoptosis to reduce subcutaneous adipose fat. A more comprehensive understanding of the precise mechanism of selective adipose apoptosis mediated by CBL-514 injections would enhance the development of lipolysis injections to overcome the limitations of existing treatments.

CONCLUSIONS

Our results show that CBL-514 is safe, well-tolerated, and effectively reduces abdominal subcutaneous fat volume and thickness at multiple doses up to 2.0 mg/cm^2. CBL-514 led to few significant adverse reactions and thus minimizes

posttreatment downtimes and the possibility of subsequent complications or sequelae. The safety and efficacy data thus support the clinical use of CBL-514 at a dose of 2.0 mg/cm^2 for optimal reduction of subcutaneous abdominal fat. Compared with the published efficacy results of existing medical treatments or procedures, and with few other alternatives for targeted, injection lipolysis, CBL-514 has potential for application as an in-clinic nonsurgical procedure to achieve more effective and localized subcutaneous fat reduction. Future studies will include further dose escalation to maximize the treatment area, optimization of treatment courses and intervals, comparisons to placebo and other products, and investigations into the fat reduction efficacy of CBL-514 in other body areas.

Supplemental Material

This article contains supplemental material located online at www.aestheticsurgeryjournal.com.

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Disclosures

Dr Goodman was the principal investigator and has been a consultant for Caliway Biopharmaceuticals (New Taipei City, Taiwan). Dr Ho consults for and holds shares in Caliway Biopharmaceuticals. Dr Chang is a director of the board for Caliway Biopharmaceuticals. Ms Ling and Ms Sheu are current employees of Caliway Biopharmaceuticals.

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