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An Uncontrolled Innovative Test of Cryo-Chilled Carbon Dioxide as A Novel Amplification of a Nano-Botanical Multi-Acting Therapy in Advanced Stage Androgenetic Alopecia (Aga)

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Abstract

Androgenetic alopecia (AGA) constitutes a progressive, polygenic, heterogenous disorder diagnosed in more than ninety percent of male and female adults presenting with scalp hair loss. While a number of treatment approaches offer limited benefit, a safe, pain free, consistently efficacious regimen remains an elusive goal. In 2020, we reported positive findings arising from a 270-day, clinical study trialing a Nano-enabled, botanically-based, oral and topical concomitant regimen dosed in two male subjects with advanced-stage AGA. As follow on to that work, this non-randomized, uncontrolled test of innovation report describes unforeseen, highly positive response to therapy observed in five AGA-affected male volunteers and one female AGA-affected volunteer tested with a cryo-carboxy dispensing device. Conceived as an augmented enhancement of a demonstrably safe and efficacious, low-risk therapy, this point-of-service tool delivered a cryo-chilled, CO2 bolstered, hydrogel solubilized iteration of the previously tested botanical formula topically to the balding scalp of enrolled volunteers, once-weekly. To limit variables, daily use oral and topical iterations of the previously trialed formula were incorporated as concomitants. Originally conceived as a nine-month (270-day) evaluation, quite unexpectedly, by day-90 a profoundly accelerated response to therapy was observed in all subjects involved, most strikingly in those initially presenting with advanced stage phenotype. Eclipsing predicted, best-case scenario endpoint outcomes, results at day-90 were judged sufficiently positive to conclude the innovative practice experiment 180 days earlier than planned. As the effort focused upon the tested device as the critical differentiator, this paper explores the history of cryo and carboxy and considers whether, in light of the outcomes observed within the tested novel innovation, a transition to generalized knowledge pursuit, as via controlled studies coupled with basic science interrogation, constitute reasonable, next step follow on.

Keywords: Hair Loss, Cryo Therapy, Carbon Dioxide Therapy, Innovative Procedure, Botanical, Androgenetic Alopecia.

Introduction

Background & Significance

With a lifetime prevalence rate greater than 50% in both males and females, androgenetic alopecia (AGA) constitutes, by far, the most common form of hair loss in all affected cohorts. While gender differences influence onset and progression, key pathophysiological features of AGA include altered hair cycle dynamics and duration, sustained follicular miniaturization, and characteristic markers of inflammation. Due to its polygenic susceptibility profile, it is now well-recognized that AGA involves multiple interactions that encompass genetics, steroid hormone metabolism and chronobiology [1].

The mainstream medical treatment of AGA, also known as common pattern hair loss, consists primarily of FDA approved drugs such as minoxidil and finasteride, each with demonstrated effica-

cy but each also linked to negative side-effects. Recent reports of cell based or biologic therapies, such as platelet rich plasma (PRP) or mesenchymal stem cell derived exosomes have also shown inconsistent results [2]. Due to its polygenic etiology, it has been suggested that AGA is unlikely to respond optimally to a single therapeutic agent or approach. Aside from mainstream medical therapy, autologous tissue graft transplant surgery and non-surgical artificial cosmesis constitute longstanding alternative patient options.

Botanical Therapy

To optimize medicant preparations, various combinations of active ingredients plus delivery-enhancing methods have been investigated [3]. Recent studies report the utility of naturally derived botanical preparations with enhanced efficacy using improved oral and topical delivery formulations [4]. As the hair follicle constitutes an ectodermal appendage that is amenable to both local and systemic therapy, our group has focused on developing concomitant oral and topical treatment strategies [5].

We previously tested topical and oral botanicals against the benchmark drug, finasteride, and demonstrated positive outcomes [6]. Subsequently, we reported the use of a novel oral and topical concomitant botanical formula dosed in two subjects with AGA, who demonstrated visible reversal of phenotype evident upon the conclusion of the study at 270 days [7-9].

Cryo-Carboxy

Both cold therapy (cryo) and carbon dioxide therapy (CO2 / carboxy) have long been used to treat multiple disease states. As represented in Table 1, several lines of evidence have emerged supporting the therapeutic benefit of cryo as well as CO2 as a method of enhancing topical penetration of active agents into the skin.

Table 1: Cryo-Carboxy / Examples Of Use (see Table 1 Reference List for noted cites)

Topic/Data	Application / Intervention	Outcomes	Study Design
1981 – Cryo-Analgesia (1)	Review of technique to block nerve transmission and provide pain relief using cryo	Temporary relief from post-operative or chronic pain	Overview of tested applications - Neuroma, Periosteum, Scars
1989 – Increased Perfusion (2)	Carbon dioxide bath as vascular optimization therapy in injured animal model	Study showed carbon dioxide bath increased blood flow in healthy or injured animal model, supports use for ulcers or wound healing	Experiment to measure CO2/H2O level against concentration-dependent increase in blood flow
2012 – Periorbital Rejuvenation (3)	Carbon dioxide to improve periorbital wrinkles and dark circles under eye	Significant improvements in wrinkles and reduction in dark circles self-reported	Twenty-patient study over seven weeks of treatment
2015 – The Bohr Effect & its emerging role within Cosmetic Dermatology (4)	Carbon dioxide water produced concentration dependent increase in blood flow	Carboxytherapy facilitates improved skin oxygenation immediately following treatment	Twelve-patient split-face study to contrast measure tcPO2 testing CO2 therapy vs microdermabrasion
2018 – Carboxytherapy for AA and AGA (5)	Placebo-controlled trial testing safety & efficacy with carboxytherapy	Statistically significant improvements in clinical score, global assessments & digitally measured findings	Two cohorts, eighty patients total (Forty AA, Forty AGA)
2018 – Scar Management (6)	Modified subcision with Carbon Dioxide insufflation treatment	Both subjects showed satisfactory results after three fortnightly sessions	Two-patient case study with post acne scar and adhesion following liposuction
2020 – Wound Healing (7)	Carbon dioxide treatment for diabetic wounds	Superior results in improving wound surface and volume compared to control group	Measured reductions in wound surface and volume
2020 – Cryotherapy for AGA (8)	Indirect use of partly ionized gas (Cold Atmospheric Plasma - CAP)	CAP treatment well tolerated with reported improvements, suggesting foundation for more extensive trials	Fourteen patients tested (four for three months, ten for six months)

Other studies indicate that cryotherapy may stimulate the activity of natural antioxidants within the skin, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, which may serve to neutralize ROS thereby protecting the tissues against oxidative stress [10]. Therapeutic improvement has been observed in several different hair disorders and reported within a series of clinical studies. For example, a clinical experiment interrogating cryotherapy using cold atmospheric plasma (CAP), reported broad clinical benefit in AGA patients - wherein fourteen patients were treated using the indirect CAP method for three months (four patients) and six months (ten patients).

The indirect CAP treatment was well-tolerated, and most patients reported improvement, with the findings essentially replicated in another, similarly structured study [11]. Tests trialing therapeutic CO2 in hair loss models have shown similarly encouraging results. For example, in one placebo-controlled carboxytherapy trial, enrolling both AGA and AA patients, positive outcomes were observed across both cohorts. This randomized study was included eighty patients divided into two groups; Group one included forty AA patients, and Group two included forty AGA patients (Group One-A and Two-A received carboxytherapy while Groups Two-A and Two-B control groups received placebo).

All cohorts were followed up monthly for three months. Results were evaluated clinically (by assessment of Severity of Alopecia Tool (SALT) score in group One, and Sinclair scale and Norwood-Hamilton scale in group Two, respectively), by investigator assessment and dermoscopy. Notably, the AA carboxy treated 'Group One-A' patients showed modest clinical improvement in SALT score after carboxytherapy - while AA placebo 'Group One-B' patients remained unchanged. At study endpoint, AGA Group Two-A patients also showed significant clinical improvement after carboxytherapy with marked increase in hair density [12].

While some regression of observed positive results was documented during the follow-up period, hair density was still judged to be significantly improved when compared to status before treatment [13]. The mechanisms of enhanced penetration from cryotherapy and carboxy therapy have been attributed historically to the Bohr's effect and possibly vascular rebound following thermal shock [13]. Quite strikingly, when CO2 is introduced subcutaneously, it rapidly diffuses at both the cutaneous and muscular microcirculatory levels, increasing micro-vasodilatation, thereby improving blood flow through direct action upon arteriole smooth muscle cells. Other data reflects recruitment and stimulation of fibroblasts as well as an increase in the quality of the extracellular matrix [14].

When measured against mainstream standard-of-care AA treatment options cryotherapy has likewise produced noteworthy data. For example, in a trial comparing against intralesional corticosteroid injection, cryotherapy treated patients evinced lower relapse rates [15]. Although cryotherapy and carboxytherapy each appear to lead to improvement in hair disorders, no studies, to our knowledge, have been reported while testing both modalities in combination. With high relevance to the work reported herein, neither cryotherapy nor carboxytherapy appear to have been studied either separately or together with botanically derived actives in hair loss affected patients.

Innovative Test Design, Materials and Methods

Study Design & Reporting

While placebo-controlled, randomized, trials remain the gold standard of clinical research, it is axiomatic that uncontrolled, non-randomized innovative procedure reports contribute important 'front-end' knowledge -- with literature published cites describing first-effort, test-of-concept experiments across a broad spectrum of phenotypic indications [16]. Furthermore, such 'first-efforts' have often led to mainstream-adopted, enhanced patient-outcome advancements, both in surgical and non-surgical subspecialties [17, 8]. Nevertheless, whether surgical or clinical in nature, it is of paramount importance that appropriately structured, best-practices are followed, documented, and reported.

Accordingly, and to ensure adherence to well-respected methodological rigor, authors here incorporated -- and closely followed -- a comprehensive, quality control Case Report checklist -- as per the Preferred Reporting of Case Series in Surgery (PROCESS) model -- subsequently optimized to fit non-surgical, uncontrolled intervention trials [8]. As shown in Supplemental Table S1, this checklist provided authors with a lucid, accurate and systematically calibrated means to track and articulate the experiment's most salient intervention variables, outcome measures, potential advantages and pitfalls -- with all steps involved predicated upon prioritizing patient-centered best practices, first and foremost.

Test Participants

To investigate the therapeutic efficacy of this novel approach, we enrolled five male participants and one female participant with moderate to advanced stage AGA. Enrolled males were classified from moderate to advanced stage Norwood Class 4 - 6 and the female volunteer was determined to manifest advanced-stage Ludwig Grade 3. Prior to initiation, informed consent was provided and executed by all subjects.

Formulations

Each of the three test formulations (topical, oral and hydrogel) contained the same set of botanically-derived compounds previously trialed in 2020 [7, 20]. All iterations were produced with the following analytical-standard-grade actives -- validated either via gas chromatograph (GC) or high performance liquid chromatograph (HPLC): betasitosterol (≥95% purity / GC; CAS Number: 83-46-5) γ linolenic acid (≥ 98.5% purity / GC; CAS Number: 506-26-3) epigallocatechin gallate (≥ 95.0% purity / HPLC; CAS Number: 989-51-5) genistein (≥ 97.0% purity / HPLC; CAS Number: 446-72-0) curcumin (≥ 97.0% purity / HPLC; CAS Number: 458-37-7) piperine (≥ 95.0% purity / HPLC; CAS Number: 94-62-2).

To optimize for potency, stability and efficient transport to the target tissue, a 2-hydroxypropylbetacyclodextrin nano delivery approach was chosen for its excellent safety profile as well as its ability to support functional amphiphilic carrier design strategies. Chromatographic analysis of the β-cyclodextrin inclusion complex resulted in a 1:1 host-to-guest stoichiometry. During final-stage production, the host–guest inclusion complex was then reduced into a fine granular powder suitable for incorporation into the three pleiotropic variants tested.

The Test Protocol

Daily therapy consisted of a single tablet (375 mg. total actives) oral bolus combined with a single application of topical formula (5 mL) to the AGA-affected scalp. Accordingly, the point-of-service cryo-carboxy delivery device, styled 'CryoTouch® MD', represented the key innovative facet of this experiment.

Cryo / Carboxy Device Preparation & Treatment Application

To optimize for delivery via the cryo-carboxy dispensing device, the botanical complex was further solubilized within an ultra-pure 2-hydroxyethyl methacrylate construct as the hydro-

gel component (Aquasonic™, Parker Laboratories, Inc.) pH 6.5 – 6.95, viscosity 130,000 – 195,000 cps. The resulting mixture was then stabilized via simple sonication. After being loaded into a polystyrene barrel cartridge, the test formula was ready for use. Designed to hold a 20 oz pre-loaded canister of medical grade carbon dioxide, the device is therefore a self-contained dispensing instrument.

For topical application, the attachable barrel cartridge may hold and deliver up to 8mL - although for this protocol, each cartridge was loaded with 5 mL of formula. Each cartridge is labeled with an RFID tag which is programed for the temperature and dispense speed. Temperature, during dispense and application, is controlled, using ambient heat within the unit to maintain the temperature above -20oC. This ensures that treated patients experience a cold but comfortable sensation at the surface of the skin. During treatment, within the applicator tip, the temperature-controlled carbon dioxide mixes with cartridge-held formula.

Functionally, when held against the skin, a slight vibration at the tip facilitates efficient application. At the same time, a small amount of pressure builds between the applicator tip and the surface of the scalp -- thus transcutaneously delivering the 'chilled C02 plus botanical formula' directly into the target hair follicles. Each weekly cryo-carboxy treatment session was administered with the participant comfortably seated in the full Fowler's position.

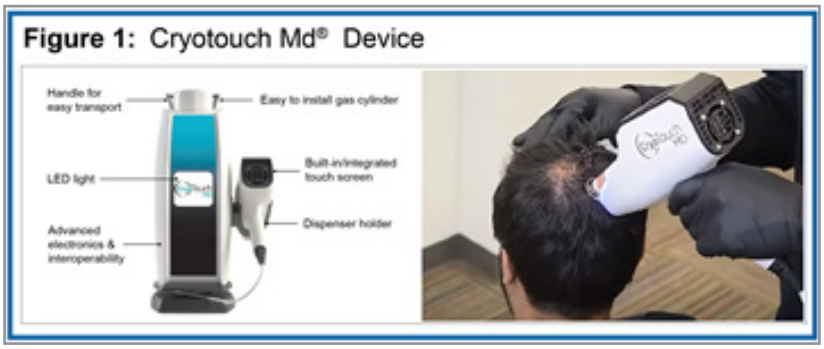


Figure 1: Crytouch Mde Device

As shown in figure 1, during therapeutic delivery the operator dispenses formula onto the scalp, working in rows while moving the applicator tip in small circles. Immediately post treatment, the scalp is then lightly blotted to remove any residual material. Enrolled participants were directed to refrain from aggressively rinsing their scalp for at least 30 minutes post-treatment -- however most allowed the formula to remain in situ for up to 24 hours. Point-of-service treatment consumed an average of five minutes - start to finish.

Measurement of Results

In order to qualitatively assess and quantitatively validate results, a number of measurements were utilized. At test day zero, the collection of gross (1x) macroscopic photos represented a primary acquisition set of baseline data, with interval progress photo capture planned consecutively -- each to occur at three 90-day time points -- throughout the initially anticipated 270-day duration of the experiment. Based on zone of hair loss, standardized images were focused upon the midfrontal vertex and / or occipital parietal scalp, with the head positioned to best visualize the AGA-affected areas.

Several subjects involved had grey or lightly pigmented hair that was not quantifiably evaluable using the operative analytic methodology, due to low pixel-capture contrast between hair and scalp. Fortuitously, two subjects had darkly pigmented hair that was evaluable by ImageJ (Male Subject J. and Female Subject C.). In these two subjects, both 1x and 10x photos were captured and quantified using the proprietary FIJI / ImageJ digital analysis platform -- and these are presented herein as representative examples of test subject response [21].

To quantify the differential hair density variance over time, histogram throughput, thusly obtained, was computed to display mean, modal, minimum and maximum shade value -- i.e. wherein greyscale variance across the x-axis is simultaneously charted against total pixel count derived from the y-axis. Once processed, and to graphically represent the differential values derived from the captured 8-bit images, the range of greyscale pixel intensity was interpolated by ImageJ analytics, sub-plotted and divided within 255 bins. Numerical value was calculated using an Excel standard mean variance algorithm [22]. A decrease in ImageJ value denotes an increase in pixel density, thereby corresponding to increased hair coverage.

Results

Patient Experience

Patient adherence with injection-based therapy has often induced compliance hesitancy, such as when measured against less invasive treatment options [23]. Moreover, long term safety concerns, particularly associated with microneedle-delivered exosome therapy, are reflected in several documented reports -- including a recently published FDA warning [24, 20]. Conversely, subjects enrolled in this innovative practice test expressed no such hesitation or concerns during this protocol, with most remarking upon the ease and lack of discomfort associated with

all facets of the regimen. Overall, tested participants reported the once-weekly, cryo/carboxy therapy application process to be quick, relaxing and pleasant.

Rapid Clinical Improvement led to early study conclusion

This test-of-innovation was initially planned as a 270-day duration, primarily based on the nine-month response-to-therapy gestation, as observed within our previously discussed work testing the same botanical composition [6, 8-9]. Quite unexpectedly, the incorporation of the once-weekly point-of-service cryo/carboxy augmentation appeared to bring about a markedly accelerated rate of regrowth that was observed across all six enrolled participants by the first evaluation time point at treatment day-90.

Therefore, at day-90 the test was concluded, enrolled participants were released, and the data was collected for analysis. No adverse events occurred during the abbreviated test period and no participant suffered the incidence of a negative side effect. In figure 2 we show 'before and after' gross macroscopic changes over the 90-day test period.

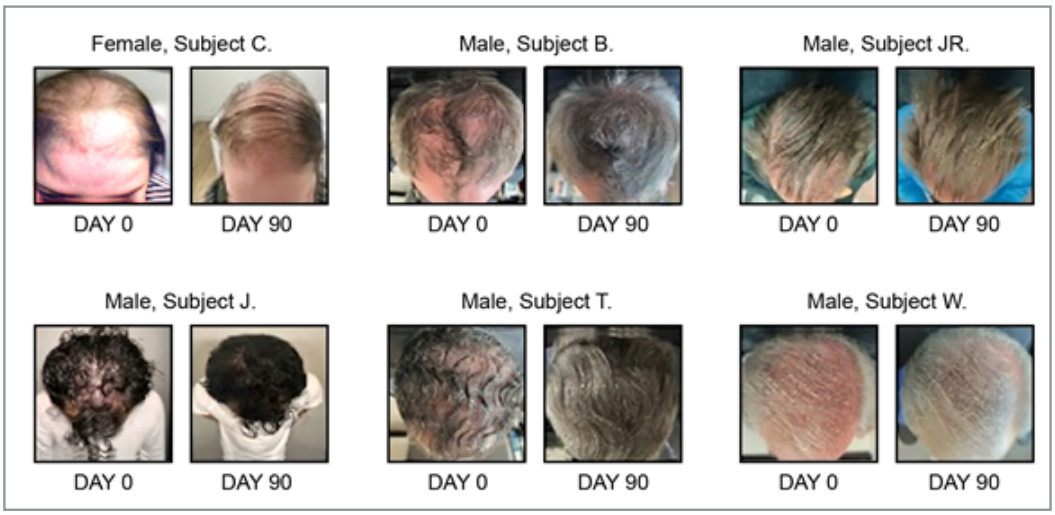


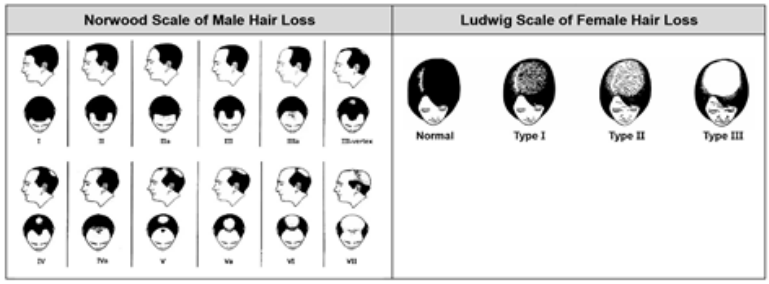
Figure 2: 90-day Gross Macroscopic Results

Qualitative Assessment of Hair Growth

In the reported work we were able to capture and show both qualitative and quantitative changes. As noted, several subjects had grey hair that was not evaluable using the quantitative methodology due to low grey-scale pixel contrast across the X and Y axes. In table 2 we grade qualitative assessment in all six subjects scaled according to observed positive improvement. Scaling was assessed with a grade of 0 = no improvement to +++ = significant improvement. Notwithstanding the abbreviated test period, we found that all enrolled subjects showed +++ significant improvement.

Table 2: 90-day Qualitatively Assessed Results

Assessment Scale		
0	=	No improvement
+	=	Mild improvement
++	=	Moderate improvement
+++	=	Marked improvement



Sex	Subject	Classification	Day 90
Female	C	LUDWIG III	+++
Male	B	NORWOOD 6	+++
Male	JR	NORWOOD 4A	+++
Male	J	NORWOOD 4	+++
Male	T	NORWOOD 5	+++
Male	W	NORWOOD 6	+++

As noted, two patients in our study had hair color to scalp contrasts that was evaluable by the FIJI / ImageJ photo analysis processing system. Therefore, we show quantitative changes in those subjects. In figure 3 (3a thru 3d) these differential changes are quantified and displayed. Global photo-macroscopic images reflect overall 90-day change at 1x magnification - while photo-micro capture contrasts 10x focal amplification of positive density change from test inception day-0 to test conclusion day-90.

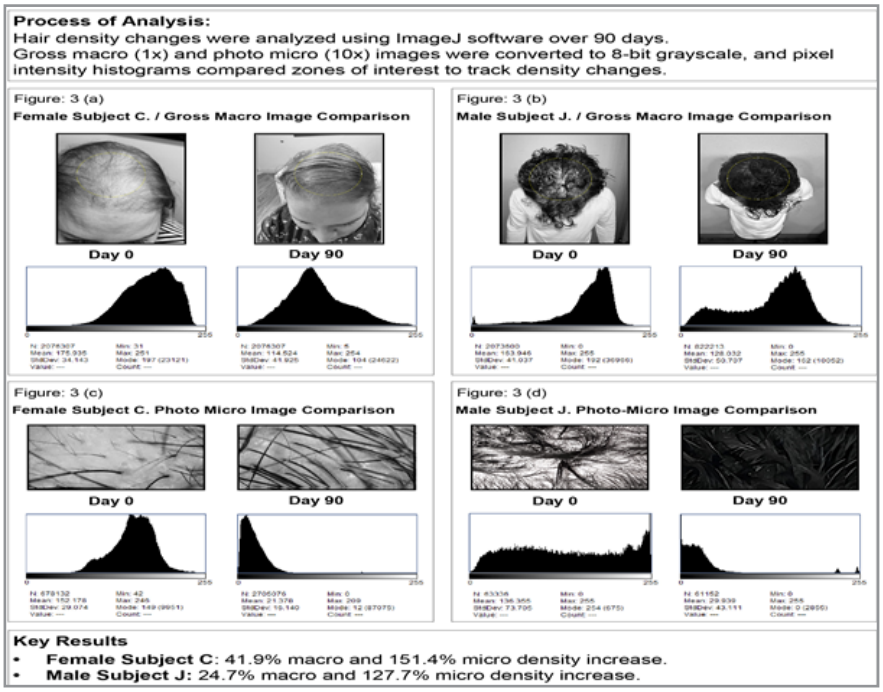


Figure 3: Comparative Analysis of Hair Density Changes

As reflected in the analyzed data, male Subject J. showed a 24.6% global macroscopic density increase at day-90 and a 127.7% photo-micro density increase over the same period. From day-0 to day-90, female Subject C. showed a 41.9% global macroscopic density increase and a 151.4% photo-micro density increase.

Discussion

It is now well-understood that the multi-factorial phenotype, androgenetic alopecia (AGA) involves complex interactions that encompass genetic susceptibility, hormonal influence and chronobiology. From a clinical perspective, as when measured against nontherapeutic small molecules, several key paradigms converge in favor of a multi-faceted, local and systemic, botanically-based approach. First, as ectodermal appendages, scalp hair follicles are amenable to concomitant oral and local therapy [25]. Second, naturally based compounds have been linked to fewer negative side effects. Third, botanically derived chemicals may be combined into a multi-faceted strategy against the polygenic phenotype, AGA [26, 27].

Challenging the paradigm, phytochemicals are intrinsically limited by poor aqueous solubility, weak permeation, low systemic bioavailability, structural instability and deactivation during first pass metabolism. Further, many promising candidate actives tested in vitro have proven highly susceptible to volatile interaction with solubilizers, excipients, buffers, co-actives and other components necessary to the development of safe, stable, efficacious, clinically appropriate medicinal agents. Thus, bridging the divide between in vitro potential and clinical utility has proven to be an elusive project [9].

Previously, we postulated that complex, pleiotropic, phytochemically-based formulations may better survive in vivo metabolic challenge when first carefully sequestered. In 2020, we observed and documented positive outcomes arising from a 270-day, clinical experiment testing a nano-enabled (hydroxypropylbetacyclodextrin invaginated), botanically-based, oral and topical, daily-use concomitant trialed in two male volunteer-subjects initially presenting with advanced-stage AGA [6, 8, 7]. The encouraging results of that work led the Authors to hypothesize as to whether further positive amplification mechanisms might be possible.

Cryotherapy and carboxytherapy each appear to have separately demonstrated published examples of safety and utility in hair disorders, yet no studies, to our knowledge appear to have tested cryo and carboxy together, or [in combination with] botanically derived actives. Therefore, a heterogenous AGA-affected cohort consisting of five males and one female -- were recruited for the purpose of evaluating a once-weekly, point of service cryo-carboxy + botanicals bolus conceived as a very low risk, non-invasive, innovative augmentation to the Authors' previously reported regimen.

Of further note, it has been observed that the use of local chemotherapy in non-melanoma skin cancers (NMSCs) has often

resulted in temporary hair loss even while using mainstream medical therapy for AGA [28]. Here, evidence for utility by cryotherapy is reported, wherein its use in the setting of NMSC sequelae blunted - or even prevented the otherwise common occurrence of transient hair loss. Such findings may perhaps point to a novel and rather intriguing mechanism - and one perhaps worthy of thoughtful inquiry [29].

Conclusions

While initially planned to mirror the previous 270-day experiment, at day-90 a strikingly accelerated rate of regrowth was observed across all enrolled participants in this test of innovative concept trial. In light of the unexpected, but highly positive response, the test was concluded 180 days earlier than originally planned -- as visible results eclipsed the predicted best-case-scenario outcomes. Accelerated regrowth was surprisingly consistent in all enrolled volunteers -- and most notably in those initially presenting with advanced stage phenotype --as illustrated by [male Subject J. / Norwood Class 4] and [female Subject C. / Ludwig Stage 3]. Here, although treated subjects were not followed post-experiment, it may be reasonably surmised that the resumption hair loss remanifested once therapy was withdrawn.

Given the unexpectedly rapid, consistently robust response to therapy attendant to this test, a reasonable explanation is called for to resolve the chasm between [anticipated and observed] findings. Here, one may perhaps conjecture that the transcutaneous delivery of cryo-carboxy plus multi-faceted, pleiotropic botanicals so improves uptake efficiency to the target tissue that time-compressed, highly positive outcomes consistently occur. Inasmuch as AGA remains a disorder with many unknowns, this is certainly a testable hypothesis – and one worthy of consideration -- as within next-step, formalized clinical trials.

In recognizing inherent weaknesses in the reported work, it must be stated that this test was constrained by its uncontrolled nature and small sample size. Likewise, although the observed results appeared to reflect an absence of any negative sequelae, extending the trial period over 270 or even 365 days, for example, may pick up on unforeseen issues not observed within the abbreviated 90-day period during which the experiment was undertaken.

Moreover, breaking out cohorts into various delivery modes (e.g. point of service cryo-carboxy delivered botanical formula alone vs point of service plus daily at-home concomitant) could perhaps provide interestingly contrasted data. Additionally, it may be valuable for the generalized knowledge to interrogate known as well as novel gene markers to assay for therapy-induced modulation.

In consequence, and to further critically assess the potential utility of the described approach, organized clinical studies – concurrent with thoughtfully conceived basic science experiments -- are strongly suggested as rational, next step follow on.

Supplemental Materials

Supplemental Table S1- CONSORT Checklist

CARE (Case Report) Checklist Adapted from 'A clinician's guide to performing a case series study' [J Bod Mov & Ther. Vol 40, p211-216. October 2024				
Item		**Yes**	**No**	**Comments**
Design	A clear rationale for the study is provided	Yes		A clear rationale for this trial articulated the compelling reason behind conducting the research... a safe, pain-free, consistently efficacious regimen remains an elusive goal.
	The research question is clearly defined	Yes		The primary hypothesis sought to examine whether carboxytherapy in combination with botanically derived actives could improve outcomes in hair loss-affected patients.
	Inclusion and exclusion criteria are clearly defined	Yes		Male and female subjects diagnosed with moderate to advanced stage androgenetic alopecia (AGA).
	The clinical setting is described	Yes		Clinical setting explained as a point-of-service, aesthetic- focused medical practice.
	Valid methods for pathology/condition identification	Yes		Diagnostic tools included Norwood scale for male subjects, Ludwig scale for female subjects, and exclusion of non- pattern hair loss phenotypes.
Intervention	The technique provided to patients is clearly described	Yes		Clear description provided about trial procedures, including purpose, duration, frequency, and risks to ensure informed participation.
	All facets of the treatment are clearly described	Yes		Comprehensive explanation of intervention protocols, including purpose, procedures, risks, benefits, and comparisons with standard treatments to aid informed decision-making.
Participants	Patient confidentiality is preserved	Yes		Measures included strict data access control, encrypted records, employee training, informed consent, secure communication, and adherence to regulations like HIPAA.

	Informed consent is provided and signed	Yes		Patients were informed about their condition, treatment options, and risks/benefits before providing consent.
Outcomes	Validated outcome measures are used	Yes		Included qualitative and quantitative analyses to track hair density changes over time.
	Values for outcome measures are clearly presented	Yes		Efforts ensured accurate data capture, making it meaningful and comparable to similar protocols.
Discussion/ Conclusion	Appropriate statistical data is reported	Yes		Measures included mean and median across an 'X', 'Y' histogram table for a logarithmic illustration of the data.
	Discussion and conclusions are supported by results	Yes		Discussion section logically follows from analyzed data presented in results, supporting interpretations and key takeaways.
Conflict of Interest	Conflicts of interest are described	Yes		Author conflicts of interest clearly articulated.

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation by S.N. Writing—review and editing, visualization, supervision and project administration by G.M. All authors have read and agreed to the published version of the manuscript.

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Ethical Review Statement

To protect the privacy and well-being, of enrolled participants, subject identities were neither requested nor provided. Authors declare that the minimal risk interventions described in the work have fully complied with the ethical principles outlined in the World Medical Association's Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013).

Informed Consent Statement

Prior to enrollment, informed consent was provided to, executed by, and obtained from all subjects involved.

Data Availability Statement

All data relevant to this work has been provided herein.

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The Authors would like to express their sincere gratitude to KC Aestheticare for generously providing access to their facilities and staff, whose support and cooperation was essential to the successful completion of this work.

Conflicts of Interest

Authors Sam Niedbala, Emma Campbell & Lincoln Young are employed by the company Cryo Concepts® LP. Author Geno Marcovici is employed by the company TrichoCyte® LLC. While acknowledging that each company could be impacted by findings of this study, the authors declare that the work was conducted independently and that their employment affiliation did not influence the results presented in this manuscript. The funding sponsors had no role in the design of the work; in the collection, analyses, and interpretation of resultant data; nor in the writing of the manuscript, or in the decision to publish the results.

References

1. Lolli, F., Pallotti, F., Rossi, A., Fortuna, M. C., Caro, G., Lenzi, A., Sansone, A., & Lombardo, F. (2017). Androgenetic alopecia, a review. Endocrine, 57(1), 9-17.
2. Nestor, M. S., Ablon, G., Gade, A., Han, H., & Fischer, D. L. (2021). Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. Journal of Cosmetic Dermatology, 20(12), 3759-3781.
3. Khezri, K., Saeedi, M., & Maleki Dizaj, S. (2018). Application of nanoparticles in percutaneous delivery of active ingredients in cosmetic preparations. Biomedicine & Pharmacotherapy, 106, 1499-1505.
4. Nasim, N., Sandeep, I. S., & Mohanty, S. (2022). Plant-derived natural products for drug discovery: Current approaches and prospects. Nucleus (Calcutta), 65(3), 399-411.
5. Chittur, S., Parr, B., & Marcovici, G. (2011). Inhibition of inflammatory gene expression in keratinocytes using a composition containing carnitine, thioctic acid, and saw palmetto extract. Evidence-Based Complementary and Alternative Medicine, 2011, 985345.
6. Chen, L., Wang, J., Mouser, G., Li, Y. C., & Marcovici, G. (2016). Blockade of androgen markers using a novel beta-sitosterol, thioctic acid, and carnitine-containing compound in prostate and hair follicle cell-based assays. Phytotherapy Research, 30(6), 1016-1020.
7. Marcovici, G., & Bauman, A. (2020). An uncontrolled case series using a botanically derived, β-cyclodextrin inclusion complex in two androgenetic alopecia-affected male subjects. Cosmetics, 7(3), 65.
8. Fleischmann, M., McLaughlin, P., Vaughan, B., & Hayes, A. (2024). A clinician's guide to performing a case series study. Journal of Bodywork and Movement Therapies, 40, 211-216.
9. Aqil, F., Munagala, R., Jeyabalan, M., & Vadhanam, M. V. (2013). Bioavailability of phytochemicals and its enhancement by drug delivery systems. Cancer Letters, 334(1), 133-141.

10. Skrzep-Poloczek, B., Romuk, E., Wisnowiska, B., Owczarek, A. J., Choreza, P., Sieron, A., Birkner, E., & Stygar, D. (2017). Effect of whole-body cryotherapy on antioxidant systems in experimental rat model. Oxidative Medicine and Cellular Longevity, 2017, 8158702.
11. Kahn, A., Malik, S., Walia, J., Fridman, G., Fridman, A., & Friedman, P. C. (2020). Tolerability of six months indirect cold (physical) plasma treatment of the scalp for hair loss. Journal of Drugs in Dermatology, 19(12), 1177-1180.
12. Dogheim, N. N., El-Tatawy, R. A., El-Hamd Neinaa, Y. M., & Abd El-Samd, M. M. (2018). Study of the efficacy of carboxytherapy in alopecia. Journal of Cosmetic Dermatology, 17(6), 945-1294.
13. Bartlett, R. G., Helmendach, R. H., & Bohr, V. C. (1953). Effect of emotional stress, anesthesia, and death on body temperature of mice exposed to cold. Proceedings of the Society for Experimental Biology and Medicine, 83(1), 4-5.
14. Draelos, Z. D., & Shamban, A. (2023). A pilot study evaluating the anti-aging benefits of a CO2-emitting facial mask. Journal of Cosmetic Dermatology, 22(8), 2198-2204.
15. Kaiser, M., Issa, N., Yaghi, M., Jimenez, J., & Issa, N. T. (2023). Review of superficial cryotherapy for the treatment of alopecia areata. Journal of Drugs in Dermatology, 22(8), 802.
16. White, A., & Ernst, E. (2001). The case for uncontrolled clinical trials: A starting point for the evidence base for CAM. Complementary Therapies in Medicine, 9(2), 111-116.
17. Matthew, G., Sohrabi, C., Franchi, T., Nicola, M., Kerwan, A., & Agha, R. (2023). Preferred reporting of case series in surgery (PROCESS) 2023 guidelines. International Journal of Surgery, 109(12), 3760-3769.
18. Patel, A., Wilcox, K., Bhinder, J., Reiser, J., & Upadhyaya, P. (2020). Low complication rates using closed-incision negative-pressure therapy for panniculectomies: A single-surgeon, retrospective, uncontrolled case series. Plastic and Reconstructive Surgery, 146(2), 390-397.
19. Luo, S., Cui, Q., & Wang, D. (2022). Case report: Surgical intervention under pheochromocytoma multisystem crisis: Timing and approach. Frontiers in Oncology, 12, 728458.
20. ImageJ. (n.d.). Learn. Retrieved from https://imagej.net/learn/
21. ImageJ. (n.d.). Fiji. Retrieved from https://imagej.net/software/fiji/.
22. ImageJ. (n.d.). Excel functions. Retrieved from https://imagej.net/plugins/excel-functions
23. Lam, W. Y., & Fresco, P. (2015). Medication adherence measures: An overview. Biomed Research International, 2015, 217047.
24. Hanyu-Deutmeyer, A., & Buchheit, T. (2023). Exosomes: The good, the bad, and the ugly. ASRA Pain Medicine News, 48. Retrieved from https://www.fda.gov/vaccines-blood-biologics/consumers-biologics/consumer-alert-regenerative-medicine-products-including-stem-cells-and-exosomes
25. Prausnitz, M., & Langer, R. (2008). Transdermal drug delivery. Nature Biotechnology, 26(11), 1261-1268. https://doi.org/10.1038/nbt1491
26. Patwardhan, B., & Gautam, M. (2005). Botanical immunodrugs: Scope and opportunities. Drug Discovery Today, 10(7), 495-502.
27. Atanasov, A. T., Zotchev, S. B., & Dirsch, V. M. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20, 200–216.
28. Izzi, S., Sorgi, P., Piemonte, P., Carbone, A., & Frascione, P. (2016). Superficial basal cell carcinoma successfully treated with ingenol mebutate gel 0.05%: Report of twenty cases. Dermatologic Therapy, 29(6), 470-472.
29. Amarillo, D., deBoni, D., & Cuello, M. (2022). Chemotherapy, alopecia, and scalp cooling systems. Actas Dermo-Sifiliográficas, 113(3), 278-283.

Table 1 References

1. Evans, P. J. D. (1981). Cryoanalgesia. Anaesthesia, 36, 1003-1013.
2. Ito, T., Moore, J. I., & Koss, M. C. (1989). Topical application of CO2 increases skin blood flow. Journal of Investigative Dermatology, 93(2), 259-262.
3. Paolo, F., Nefer, F., Paola, P., & Nicolo, S. (2012). Periorbital area rejuvenation using carbon dioxide therapy. Journal of Cosmetic Dermatology, 11, 223-228.
4. Seidel, R., & Moy, R. (2015). Effect of carbon dioxide facial therapy on skin oxygenation. Journal of Drugs in Dermatology, 14(9), 976-980.
5. Doghaim, N. N., El-Tatawy, R. A., El-Hamd Neinna, Y. M., & Abd El-Samd, M. M. (2018). Study of the efficacy of carboxytherapy in alopecia. Journal of Cosmetic Dermatology, 17(6), 1275-1285.
6. Lee, G., & SK. (2018). Carbon dioxide assisted subcision in the treatment of adherent localized scars. Journal of Surgery, 6(3), 78-81.
7. Macura, M., Frangez, H. B., Cankar, K., Finzgar, M., & Frangez, I. (2020). The effect of transcutaneous application of gaseous CO2 on diabetic chronic wound healing: A double-blind randomized clinical trial. International Wound Journal, 17, 1607-1614.
8. Kahn, A., Malik, S., Walia, J., Fridman, G., Fridman, A., & Friedman, P. C. (2020). Tolerability of six-month indirect cold (physical) plasma treatment of the scalp for hair loss. Journal of Drugs in Dermatology, 19(12), 1177-1180.