Hair Regrowth Following a Wnt- and Follistatin-Containing Treatment: Safety and Efficacy in a First-in-Man Phase 1 Clinical Trial

Michael P. Zimber PhD,[a] Craig Ziering DO,[b] Fraink Zeigler,[a] Mark Hubka DC,[a] Jonathan N. Mansbridge PhD,[a] Mark Baumgartner,[a] Kelsea Hubka,[a] Robert Kellar PhD,[a] David Perez-Meza MD,[c] Neil Sadick MD,[d] Gail K. Naughton PhD[a]

[a]Histogen Inc., San Diego, CA
[b]Ziering Medical, West Hollywood, CA
[c]Permanent Hair Solutions Consulting, Maitland, FL
[d]Sadick Dermatology, New York, NY

ABSTRACT

Research has shown the importance of follistatin, Wnt 7a, and wound healing growth factors on the stimulation of bulge cells and inter-follicular stem cells to induce hair growth. We have studied the effects of a bioengineered, non-recombinant, human cell-derived formulation, termed Hair Stimulating Complex (HSC), containing these factors to assess its hair growth activity in male pattern baldness. HSC showed in vitro Wnt activity and contained follistatin, KGF, and VEGF. The clinical study was a double-blind, placebo-controlled, randomized single site trial and was designed to evaluate safety of the HSC product and assess efficacy in stimulating hair growth. All 26 subjects tolerated the single, intradermal injection of HSC procedures well, and no signs of an adverse reaction were reported. Histopathological evaluation of the treatment site biopsies taken at 22 and 52 weeks post-treatment revealed no abnormal morphology, hamartomas, or other pathological responses. Trichoscan image analysis of HSC-treated sites at 12 and 52 weeks showed significant improvements in hair growth over the placebo. At the initial 12-week evaluation period, HSC-treated sites demonstrated an increase in hair shaft thickness (6.3%±2.5% vs. -0.63%±2.1%; P=0.046), thickness density (12.8%±4.5% vs. -0.2%±2.9%; P=0.028), and terminal hair density (20.6±4.9% vs. 4.4±4.9%; P=0.029). At one year, a statistically significant increase in total hair count (P=0.032) continued to be seen. These results demonstrate that a single intradermal administration of HSC improved hair growth in subjects with androgenetic alopecia and is a clinical substantiation of previous preclinical research with Wnts, follistatin, and other growth factors associated with wound healing and regeneration.

J Drugs Dermatol. 2011;10(11):1308-1312.

INTRODUCTION

Androgenetic alopecia is a widespread cosmetic and medical disorder for which there exist few treatment options. The current therapeutic strategies including surgical, pharmaceutical, and cosmetic interventions are limited in approach and success. We have developed a bioengineered human cell-derived formulation, termed Hair Stimulating Complex (HSC) that consists of a number of human growth factors and morphogens recognized to be critical to the induction and maintenance of hair follicle growth and activity. Here we report that the preparation is safe as applied and showed effectiveness in stimulating hair growth following the clinical administration of HSC to men with male-pattern baldness in a first-in-human, phase 1 clinical study.

In the HSC manufacturing process, neonatal dermal fibroblasts, which are closely related to hair follicle dermal papilla cells, are seeded onto microcarrier beads and grown in suspension culture under hypoxic conditions that simulate the embryonic environment. Under these conditions, the cells differentially express over 5,000 genes compared to cells grown in normoxic environments. Several of the upregulated genes expressed in the hypoxic cells are associated with pluripotent and follicular stem cells including LnX2, SOX21, Nestin, NFATc1, Krt15, POU5F1 (OCT4), SOX2 and Nanog. In addition, WNT7a, VEGF, FGF, KGF and follistatin are upregulated in these cells. In the adult, Wnt proteins have been found to play an essential role in induction of the dermal papilla[1,2] and triggering of stem cell activity in keratinocytes[3] to produce new hair follicles and growth. Follistatin is an antagonist of activin and BMP, which are involved in maintaining a slow cycling stem cell phenotype in resting hair follicles.[4] The exogenous administration of Wnt proteins and follistatin to the scalp represents a novel and practical way to ameliorate and reverse androgenetic alopecia and other related hair loss disorders. The presence of Wnt proteins

in the HSC used in these studies was assessed by immunoblot analysis using a primary antibody recognizing the Wnt7a protein (Santa Cruz Biologicals, CA) (Figure 1a). The canonical Wnt bioactivity of HSC was confirmed by demonstrating nuclear translocation of β-catenin in human epidermal keratinocytes in vitro (Figure 1b). LiCl was used as a positive control,[5] and the Wnt receptor antagonist DKK-1 prevented β-catenin translocation. The HSC solution also contained VEGF, follistatin, and KGF, as determined by ELISA (Figure 1c).

Preclinical studies demonstrated no safety issues and suggested that the induction of anagen in telogen follicles in a murine model of hair growth might be accelerated by injection of HSC.[6,7] A pilot, clinical study was then undertaken to assess the safety and efficacy of HSC in man. The study design was a single site, double-blind, randomized, placebo-controlled, clinical trial. Two HSC preparations were evaluated in the study; a 10x concentrated, serum-free preparation, and a second, non-concentrated, bovine serum containing preparation. Also as part of the study, we evaluated whether additional stimulation to the scalp prior to HSC injection would have any effect. Three different devices were used to stimulate the scalp: 1) microdermabrasion (MegaPeel, DermaMed Intl, Inc., Lenni, PA), 2) overlapping passes of nonablative 1540 and ablative 2940 erbium laser (Palomar Medical Technologies, Inc., Burlington, MA), 3) low level light therapy by the Revage670 (Apira Science Inc., Newport Beach, CA). After obtaining informed consent, 26 healthy, male subjects between 18–55 years of age were enrolled. Inclusion criteria included a Fitzpatrick score of I–IV, Norwood/Hamilton Classification for male pattern hair loss 4–6, and no history of prior hair treatments or immunological compromise. The selected study participants were randomly assigned to one of the three stimulation methods. Four zones, two anterior and two posterior, were selected as treatment sites on each subject's scalp and marked by a tattoo on its periphery to identify location. The anterior two sites were randomized right/left and injected with one of the two HSC preparations or placebo (unconditioned medium) with no pre-injection stimulation (Figure 1d). The posterior treatment sites were stimulated using one of the three treatments mentioned above followed immediately by injection with HSC or placebo, also randomized right/left. In all subjects, baseline measurements were obtained and each site received four evenly placed intradermal 0.1 cc injections. The ability to randomize and provide different, independent treatment at each of the four sites on each subject is a unique and advantageous clinical paradigm in evaluating hair restoration strategies. In addition to comparing treatment and placebo effects throughout the study in individual subjects, thereby providing in-subject control, pretreatment baseline measurements provides greater statistical power by enabling a repeated-measures experimental design. In six patients, punch biopsies (4 mm) were performed at baseline and week 22 and were used for histopathological evaluation. Biopsies were taken from four further patients at an optional one year visit. Safety outcomes were also measured through visual examination by the clinician at each time point for inflammation, redness, edema, itching, burning, swelling and any other adverse events. Global and macro photography was reviewed by independent dermatologists for any observable adverse events including redness, swelling and ingrown hairs. The biological and clinical effects of treatment were evaluated through FotoFinder Xpert Clinical Trial System (FotoFinder Systems, Columbia, MD) image acquisition and TrichoScan analysis for anagen:telogen ratios, hair diameter, hair density, hair thickness, and vellus and nonvellus, terminal hair counts. Results are expressed as group means + SEM. The equivalence of sites at baseline, prior to treatment, was determined using one-way ANOVA. Subsequent differences between group means within a treatment group at 12, 22, and 52 weeks post-treatment were evaluated using paired (repeated-measures), two-tailed Student's t-tests. All other differences between group means were evaluated using unpaired, two-tailed Student's t-tests. Statistical significance was set at level of α=0.05. A comparison of the untreated and treated baseline mean hair measurements detected no statistically significant differences, so any subsequent differences could be inferred to be the result of experimental treatment. A combination of investigator global assessments and test subject self-assessments were also performed at the times of clinical visits. Investigators used a rating system to determine visual improvement in regional hair growth.

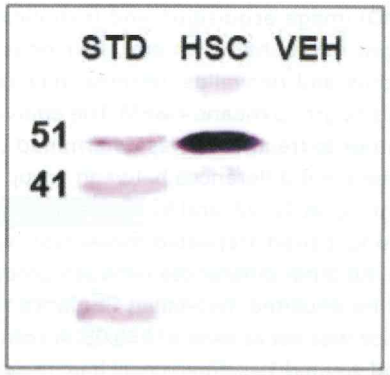
DISCUSSION

The primary objective of the study was to assess clinical safety of HSC administration in human subjects. All patients tolerated the procedures well and no significant complaints, clinical symptoms or signs of an adverse reaction were reported in any subjects. The histopathological evaluation of the treatment site biopsies taken at baseline, 22 weeks, and one year post-treatment revealed minimal to slight inflammation at the injection site and no abnormal morphology, hamartomas, or other pathological responses. From these observations, it is concluded that a single, intradermal injection of HSC did not result in any significant toxicity, pathology or other adverse event during the course of the study.

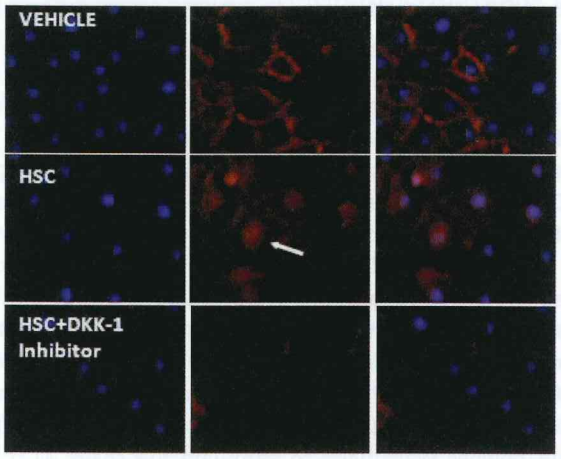
For the secondary objective, to assess the clinical effect of HSC, the sites treated with 10x, serum-free HSC (n=13) demonstrated an increase in most hair growth indicators over the initial 12-week evaluation period. A statistically significant increase from baseline values in the hair shaft thickness (P=0.04), hair thickness density (P=0.025) and the number of nonvellus, terminal hairs (P=0.003) was observed in the HSC treated sites (Figure 1e). The improvements caused by HSC treatment were significantly greater than those observed in placebo-treated sites for hair shaft thickness (6.3%±2.5% vs. -0.63%±2.1%; P=0.046), thickness density (12.8%±4.5% vs. -0.2%±2.9%; P=0.028), and terminal

FIGURE 1. Clinical effect and characterization of HSC. **a)** HSC contained Wnt7a and growth factor proteins as detected by immunoblot. **b)** Wnt bioactivity was verified by observing nuclear translocation of β-catenin (arrow) in human keratinocytes following HSC treatment, which was blocked by the Wnt receptor antagonist DKK-1. **c)** Follistatin, KGF, and VEGF concentrations in HSC preparation as determined by ELISA. **d)** Diagram illustrating the randomized, experimental design of HSC administration and stimulation method (Stim.) of the four treatment sites in each subject. See text for details. **e)** HSC treatment caused a significant increase in hair shaft diameter, hair density, and the number of nonvellus, terminal hair growth at three months following a single intradermal injection as compared to control treatment (*P<0.05). **f)** Subject 027 showed a 123.4% increase in total hair count at one year following a single injection of HSC. In-subject control site increased only 4.8%. Scale bar=2 mm.

a)



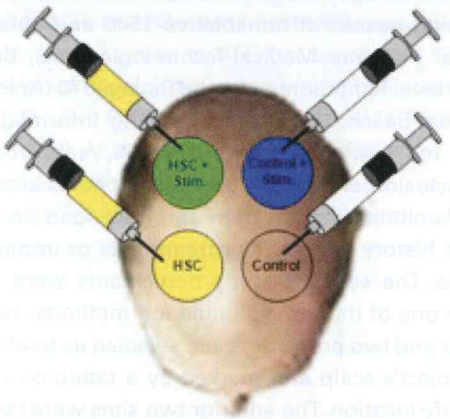
b)



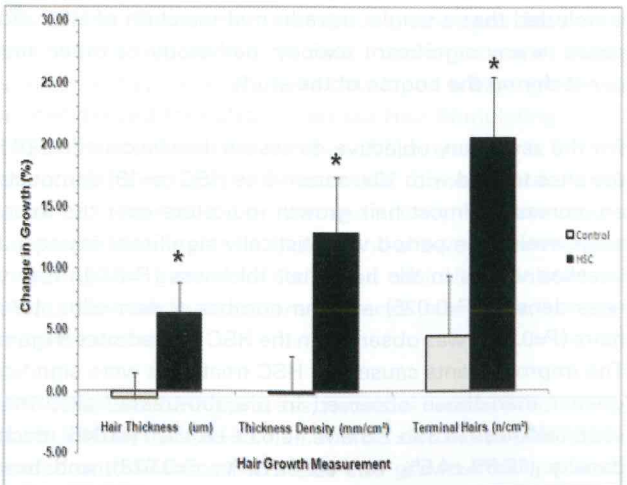
c)

Protein	[ng/ml]
Follistatin	219.7
KGF	4.1
VEGF	121.0

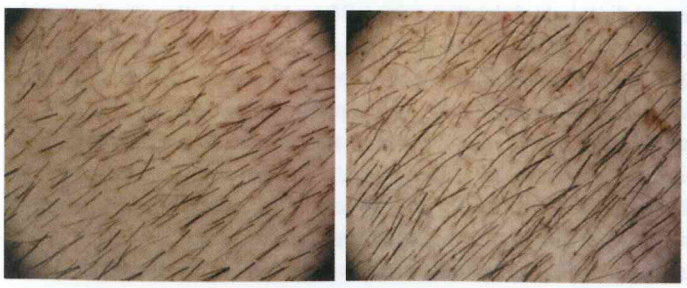
d)



e)



f)



S1027 Right Anterior Baseline
214.5 Hairs

S1027 Right Anterior 1 year
324.5 Hairs

hair density (20.6±4.9% vs. 4.4±4.9%; P=0.029). Although a similar trend was seen at 22 weeks, significance was lost as there was no further growth improvement in the subjects. However, at one year, we observed significant improvements in hair count (16.0±6.6% vs. 3.65±3.7%) and substantial increases in thickness density (17.6±8.39% vs. 0.67±4.3%), and terminal hair density (29.5±14.8 vs. 2.4±6.8%). Figure 1f illustrates the hair growth improvement observed at 12 months after HSC administration in a single patient. Placebo treated sites (n=12) at 12, 22, and 52 weeks showed no significant improvements in any of the measured hair growth indicators. In addition, no significant effects were seen with the serum containing HSC preparation. The additional scalp stimulation prior to the HSC administration did not result in an enhancement in growth from that of the serum-free HSC administration alone. In order to analyze the distance over which HSC demonstrated efficacy in relation to the injection site, the distribution of hair density in the treated area was averaged over 48 quadrants. The distribution plot suggested that HSC's effect on hair growth was concentrated within 1–2 mm of the site of injection and at a point central to all four injection sites. These results are consistent with publications noting a limited diffusion of Wnt and somewhat greater diffusion of follistatin. These results clearly demonstrate that a single intradermal administration of 10x HSC significantly improved hair growth in subjects with androgenetic alopecia.

CONCLUSION

This first-in-man clinical study demonstrates the safety of the cell-secreted HSC protein preparation and is an initial indication of efficacy in hair restoration. The HSC proteins produced by the skin cells grown under hypoxic conditions include factors that are important in hair cycle regulation. In vitro studies have demonstrated the presence of Wnt proteins, VEGF, FGF, KGF and also follistatin. The mechanisms responsible for hair growth have been studied for decades as researchers have demonstrated the underlying importance of Wnt proteins and wound growth factors in stimulating dermal papilla-associated stem cells.[8] Wnt proteins play a crucial role in the later stages of telogen in the initiation of hair growth,[2,9,10] activating cells and gene expression required for the formation of a hair germ that will commence the next anagen.[11] Therefore, it is hypothesized that the stimulation of hair regrowth induced by the delivery of HSC is due, at least in part, to Wnt activity. Concurrently follistatin relieves the inhibitory action of BMP2 and activin on hair follicle stem cell proliferation. Wound healing associated growth factors, KGF and VEGF, stimulate keratinocyte proliferation in the developing hair follicle and local angiogenesis to promote hair development.

The efficacy results seen with a single injection of HSC represent a novel approach in hair growth treatment. FDA approved products, minoxidil (e.g., Rogaine) and finasteride (e.g., Propecia), require daily use of the therapeutic to induce and maintain efficacy.[12-15] Specifically, these products show their greatest efficacy in reducing loss of hair, with a small percentage of new hair growth seen after at least four months of daily use. In contrast, a single injection of HSC resulted in a statistically significant growth of new terminal hairs and an increase in hair density and thickness at 12 weeks that was still detectable after one year. In addition, the hair growth was limited to the region surrounding the injection, suggesting that HSC may provide long term and site-controlled efficacy. This clinical pilot study is the first demonstration that a preparation containing Wnt proteins and growth factors has a biologically active stimulatory effect on new hair induction in humans.

This first-in-man clinical study demonstrates the safety of the cell-secreted HSC protein preparation and is an initial indication of efficacy in hair restoration.

ACKNOWLEDGEMENTS

We should like to thank Dr. Lior Rosenberg-Belmaker, Dr. David Whiting, Dr. Rolf Hoffmann, Dr. Melissa Carpenter, and Dr. Nelly Paz for experimental data, interpretation of pathology, organization of the clinical trial and advice.

DISCLOSURES

The following authors are employees or consultants of Histogen: Dr. Michael Zimber, Frank Ziegler, Dr. Mark Hubka, Dr. Jonathan Mansbridge, Mark Baumgartner, Kelsea Hubka, Dr. Robert Kellar, Dr. Gail Naughton. The following authors are members of the Histogen Scientific Advisory Board: Dr. Craig Ziering, Dr. Neil Sadick.

REFERENCES

1. Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev.* 2000;14:1181-1185.
2. Greco V, Chen T, Rendi M et al. A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell.* 2009;4:155-169.
3. Ito M, Yang Z, Andl T, et al. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. *Nature.* 2007;47:316-320.
4. Nakamura M, Matzuk MM, Gerstmayer B, et al. Control of pelage hair follicle development and cycling by complex interactions between follistatin and activin. *FASEB J.* 2003;17:497-499.
5. Kashour T, Burton T, Dilbrov A, Amara F. Myogenic signaling by lithium in cardiomyoblasts is Akt independent but requires activation of the beta-catenin-Tcf/Lef pathway. *J Mol Cell Cardiol.* 2003;35:937-951.
6. Maurer M, Handjiski B, Paus R. Hair growth modulation by topical immunophilin ligands: Induction of anagen, inhibition of massive catagen development, and relative protection from chemotherapy-induced alopecia. *Am J Pathol.* 1997:150:1433-1441.