Effect of Propecia on the Hair Follicle in Male Androgenetic Alopecia: A Confocal Laser Scanning Microscopy and Video Imaging Study
Investigators: Department of Dermatology, University of Minnesota, Minneapolis, Minnesota, USA

Maria Hordinsky, MD
Marna Ericson, PhD
Cathy Boeck, RN, DNR, CCRC
Dawn Snow, LPN, CCRC
Jill Plumb-Smith, BA
Christine Baker, BS

Medical School Grants Program, Merck & Co., Inc.
**Objective**

Characterize changes in hair follicle innervation, vasculature, and melanocytes before and after administration of Propecia (1 mg/day) or placebo for 9 months.
## Study Design

<table>
<thead>
<tr>
<th>Patients</th>
<th>15 Caucasians and 1 African-American with Hamilton type IV or V balding, between the ages 18-41 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Propecia 1 mg po qd or placebo, 1 tablet po qd, for nine months</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Clinical photographs, investigator assessment, analysis of 4-mm scalp punch biopsies by H &amp; E and LSCM</td>
</tr>
<tr>
<td>Analysis of</td>
<td>Immunohistochemistry and laser scanning confocal microscopy (LSCM)</td>
</tr>
<tr>
<td>nerves, vessels, and melanocytes</td>
<td></td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>15 Caucasians and 1 African-American with Hamilton type IV or V balding, between the ages 18-41 years</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Propecia 1 mg po qd or placebo, 1 tablet po qd, for nine months</td>
</tr>
<tr>
<td><strong>Efficacy</strong></td>
<td>Clinical photographs, investigator assessment, analysis of 4-mm scalp punch biopsies by H &amp; E and LSCM</td>
</tr>
<tr>
<td><strong>Analysis of nerves, vessels, and melanocytes</strong></td>
<td>Immunohistochemistry and laser scanning confocal microscopy (LSCM)</td>
</tr>
</tbody>
</table>
# Study Design

<table>
<thead>
<tr>
<th>Patients</th>
<th>15 Caucasians and 1 African-American with Hamilton type IV or V balding, between the ages 18-41 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Propecia 1 mg po qd or placebo, 1 tablet po qd, for nine months</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Clinical photographs, investigator assessment, analysis of 4-mm scalp punch biopsies by H &amp; E and LSCM</td>
</tr>
<tr>
<td>Analysis of nerves, vessels, and melanocytes</td>
<td>Immunohistochemistry and laser scanning confocal microscopy (LSCM)</td>
</tr>
<tr>
<td>Study Design</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>15 Caucasians and 1 African-American with Hamilton type IV or V balding, between the ages 18-41 years</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Propecia 1 mg po qd or placebo, 1 tablet po qd, for nine months</td>
</tr>
<tr>
<td><strong>Efficacy</strong></td>
<td>Clinical photographs, investigator assessment, analysis of 4-mm scalp punch biopsies by H &amp; E and LSCM</td>
</tr>
<tr>
<td><strong>Analysis of nerves, vessels, and melanocytes</strong></td>
<td>Immunohistochemistry and laser scanning confocal microscopy (LSCM)</td>
</tr>
</tbody>
</table>
Pre- and Post-Treatment Analyses

1. Clinical photographs
   1. Vertex
   2. Anterior

2. Histology

3. Investigator assessment

4. LSCM merged z-series images of multi-stained biopsy sections
Pre-Treatment

Hamilton Type IV

Post-Treatment

Hamilton Type IV

Placebo +/-06B
Pre-Treatment

Post-Treatment

Placebo +/-06B
Pre-Treatment

Hamilton Type IV

Propecia +/-01A

Post-Treatment

Hamilton Type III
Pre-Treatment

Post-Treatment

Propecia +/-01A
Pre- and Post-Treatment Analyses

1. **Clinical photographs**
   1. Vertex
   2. Anterior

2. **Histology**

3. **Investigator assessment**

4. **LSCM merged z-series images of multi-stained biopsy sections**
OBSERVATION: Follicles deeper in dermis/fat

Propecia +/-01A
Pre- and Post-Treatment Analyses

1. Clinical photographs
   1. Vertex
   2. Anterior

2. Histology

3. Investigator assessment

4. LSCM merged z-series images of multi-stained biopsy sections
   • vertically-sectioned
   • 200 – 300 microns thick
Immunohistochemical Epifluorescent Techniques

- **Nerve marker**: Polyclonal antibody to pan-neuronal cytoplasmic protein gene product 9.5 (PGP 9.5) and anti-rabbit Cy 5 or Cy 3

- **Melanocytes**: Monoclonal antibody Mel 5 and anti-mouse Cy 3 or Cy 5

- **Vasculature and follicular keratinocytes**: Fluorescein-conjugated lectin *Ulex europaeus* agglutinin (UEA1-FITC)
Laser Scanning Confocal Microscopy (LSCM)

LSCM differs from conventional wide-field microscopy because:

- Out-of-focus epifluorescent light is excluded, thereby increasing the contrast and resolution in the plane of focus.
- Images from defined levels, in the z-plane of focus, can be captured and stored in the computer's memory and projected in register.
- Thick sections (up to 400 microns) can be imaged so that the entire, intact hair follicle is captured.
Laser Scanning Confocal Microscopy (LSCM) Captures 3-Dimensional Data of Intact Cellular Structures and Interactions

Bottom optical section in z-plane of sample.

Confocal microscope records digital ‘image’ of each optical section.

Top optical section in z-plane of sample.

Digitized layers merged into single ‘in-focus’ projection.
examples
Normal Scalp
Anagen Hair Follicle
Montage of four LSCM z-series fields

Normal Scalp
Anagen Hair Follicle
Montage of four LSCM z-series fields

Normal Scalp
Anagen Hair Follicle

PGP 9.5 antibody labels nerves
Montage of four LSCM z-series fields

Normal Scalp
Anagen Hair Follicle

PGP 9.5 antibody labels nerves

UEA1-FITC stains:
- blood vessels
- follicular keratinocytes
- subset of epidermal keratinocytes
Bulge region of normal anagen scalp hair follicle.
Bulge region of normal anagen scalp hair follicle.

*PGP9.5/Nerves*
Bulge region of normal anagen scalp hair follicle
Bulge region of normal anagen scalp hair follicle.
Bulge region of normal anagen scalp hair follicle.
Bulge region of normal anagen scalp hair follicle.

**PGP9.5/Nerves**

**CGRP/Neuropeptide**
UEA1-FITC Staining of Perivascular Network Surrounding Bulb Region of Normal Scalp Anagen Hair Follicle
UEA1-FITC Staining of Vasculature and Follicular Keratinocytes in and around Dermal Papilla of Normal Scalp Anagen Hair Follicle
Pre- and Post-Treatment Analyses

1. Clinical photographs
   - Vertex
   - Anterior

2. Histology

3. Investigator assessment

4. LSCM merged z-series images
   - Melanocytes – blue color
   - Vessels and follicles – red color
   - Nerves – green color
Pre-Treatment
Pre-Treatment

Merged LSCM
z-Series of Multi-Stained Scalp Biopsy
Pre-Treatment

Miniaturized Follicles

Merged LSCM z-Series of Multi-Stained Scalp Biopsy
Pre-Treatment

Miniaturized Follicles

Merged LSCM
z-Series of Multi-Stained Scalp Biopsy

area of higher magnification
Pre-Treatment

Merged LSCM
z-Series of Multi-Stained Scalp Biopsy
Pre-Treatment
Pre-Treatment

UEA1-FITC stains:
• blood vessels
• follicular keratinocytes
• subset of epidermal keratinocytes
Pre-Treatment

*PGP 9.5 antibody labels nerves.*
Pre-Treatment
Pre-Treatment

*Mel 5 antibody labels epidermal and follicular melanocytes.*
Pre-Treatment

3-D Animation of Merged LSCM z-Series
Post-Treatment
Post-Treatment

Merged LSCM
z-Series of Multi-Stained Scalp Biopsy
Post-Treatment

area of higher magnification
Innervation of Putative Stem Cell/Bulge Region.

Post-Treatment
Summary

• Propecia induced hair regrowth.

• After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  – enlarged follicle diameters
  – follicles deeper in dermis/fat
  – perifollicular innervation comparable to normal
  – Mel5 antibody staining in the dermal papilla comparable to normal
Summary

• Propecia induced hair regrowth.

• After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  – enlarged follicle diameters
  – follicles deeper in dermis/fat
  – perifollicular innervation comparable to normal
  – Mel5 antibody staining in the dermal papilla comparable to normal
Summary

• Propecia induced hair regrowth.

• After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  – enlarged follicle diameters
  – follicles deeper in dermis/fat
  – perifollicular innervation comparable to normal
  – Mel5 antibody staining in the dermal papilla comparable to normal
Summary

- Propecia induced hair regrowth.
- After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  - enlarged follicle diameters
  - follicles deeper in dermis/fat
  - perifollicular innervation comparable to normal
  - Mel5 antibody staining in the dermal papilla comparable to normal
Summary

- Propecia induced hair regrowth.

- After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  - enlarged follicle diameters
  - follicles deeper in dermis/fat
  - perifollicular innervation comparable to normal
  - Mel5 antibody staining in the dermal papilla comparable to normal
Summary

- Propecia induced hair regrowth.

- After nine months Propecia therapy, LSCM images of multi-stained biopsies revealed:
  - enlarged follicle diameters
  - follicles deeper in dermis/fat
  - perifollicular innervation comparable to normal
  - Mel5 antibody staining in the dermal papilla comparable to normal

...qualitative
Continued Analysis

Quantitative analysis of melanocytes in hair bulb

– all subjects,
– before and after treatment,
– using stereological analysis of three-dimensional LSCM z-series images.
Transplant Proposal

- Demonstrate that nerves and blood vessels in transplanted hair follicles become integrated into the dermal nerve and vasculature plexus of the recipient site, respectively, after 3 months post surgery.

- Demonstrate increased VEGF, FKBP12, SP, and NGF in and around the transplanted hair follicles at 2 weeks post surgery versus pre-surgery and 3 months post-surgery.
Transplant Proposal

- Demonstrate that nerves and blood vessels in transplanted hair follicles become integrated into the dermal nerve and vasculature plexus of the recipient site, respectively, after 3 months post surgery.

Structure

- Demonstrate increased VEGF, FKBP12, SP, and NGF in and around the transplanted hair follicles at 2 weeks post surgery versus pre-surgery and 3 months post-surgery.
Transplant Proposal

- Demonstrate that nerves and blood vessels in transplanted hair follicles become integrated into the dermal nerve and vasculature plexus of the recipient site, respectively, after 3 months post surgery.

Structure

- Demonstrate increased VEGF, FKBP12, SP, and NGF in and around the transplanted hair follicles at 2 weeks post surgery versus pre-surgery and 3 months post-surgery.
Transplant Proposal

• Demonstrate that nerves and blood vessels in transplanted hair follicles become integrated into the dermal nerve and vasculature plexus of the recipient site, respectively, after 3 months post surgery.

Structure

• Demonstrate increased VEGF, FKBP12, SP, and NGF in and around the transplanted hair follicles at 2 weeks post surgery versus pre-surgery and 3 months post-surgery.

Growth Factors and Endogenous Neurotrophic Compounds