TOPICAL TREATMENT OF ACTINIC KERATOSIS

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Conflicts of Interest Disclosure

- Abbvie
- Allergan
- Amgen
- Anacor
- Aqua
- Bayer
- Castle
- Celgene
- Dermira
- Eclipse
- Galderma
- Genentech
- GSK/Stiefel
- Intraderm
- ISDIN

- Janssen
- Leo
- Menlo
- Novartis
- Pharmaderm
- Pfizer
- Ranbaxy
- Regeneron
- Scibase
- Suneva
- TEVA
- Valeant/Ortho Dermatologica
- Verrica
- Xoft

- Digital editor for CUTIS
Outline

• Why treat AK?
  – Progression to SCC
  – Field cancerization

• Timed Sequential Therapy (TST) with
  – Imiquimod 3.75%
  – Ingenol mebutate
  – 5-FU 0.5%
  – Photodynamic therapy
Gene Expression Patterns of Normal Human Skin, Actinic Keratosis, and Squamous Cell Carcinoma

A Spectrum of Disease Progression

R. Steven Padilla, MD, MBA; Sheldon Sebastian, MD; Zeyu Jiang, PhD; Ingo Nindl, PhD; Richard Larson, MD, PhD

Objectives: To identify and compare the gene expression profiles of actinic keratosis (AK) and squamous cell carcinoma (SCC) and to further clarify critical genetic alterations in the evolution of SCC from normal sun-damaged human skin.

Design: Observational study.

Setting: University practice.

Patients: Skin biopsy specimens were obtained from 16 patients. The specimens included 14 normal non-sun-exposed skin samples, 14 normal sun-exposed skin samples, 5 AKs, and 15 cutaneous SCCs.

Main Outcome Measures: Gene expression profiles from normal non-sun-exposed skin, normal sun-exposed skin, AKs, and SCCs.

Results: Using a highly astringent shrunken centroid threshold of 6.52 and the prediction analysis of microarrays, we identified 89 unique genes that most likely contribute to the molecular evolution of SCC. Our model was cross-validated using data from a separate study and clearly distinguishes between skin tumors (AK and SCC) and normal skin independent of sun exposure. Genes that were upregulated in AK and SCC were downregulated in normal skin, and genes that were downregulated in AK and SCC were upregulated in normal skin.

Conclusions: The finding of similar differentially expressed genes in AK and SCC confirms that AK is a precursor lesion of SCC and indicates that they are closely related genetically. Clear elucidation of these relationships will be critical to improving therapeutic approaches.

Arch Dermatol. 2010;146(3):288-293
Actinic Keratosis

• Larson and colleagues examined
  – 14 normal non–sun-exposed skin samples
  – 14 normal sun-exposed skin samples
  – 5 AKs
  – 15 cutaneous SCCs
  – High-density gene microarray studies were performed on isolated RNA samples

Actinic Keratosis

• Direct correlation of abnormal gene expression in the progression of normal skin to AK to SCC

Actinic Keratosis

- 186 genes statistically significant
  - 101 - expressed progressively higher along the spectrum
  - 85 - expressed progressively lower along this spectrum of disease
  - AK and SCC - most evident degree of gene expression
    - Normal skin - minimal alteration of gene expression

Actinic Keratosis - Follicular Extension

• Examined 1000 AKs
  – 104 with follicular extension
  – Determine the prognostic significance of follicular extension in AK

Actinic Keratosis - Follicular Extension
Actinic Keratosis - Follicular Extension

Table 1: Cancer occurrences versus history of actinic keratosis with follicular extension.

<table>
<thead>
<tr>
<th></th>
<th>Squamous cell carcinoma</th>
<th>Basal cell carcinoma</th>
<th>Melanoma</th>
<th>No cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>With follicular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extension</td>
<td>64 (52%)</td>
<td>40 (54%)</td>
<td>11 (61%)</td>
<td>30 (41%)</td>
</tr>
<tr>
<td>Without</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>follicular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extension</td>
<td>60 (48%)</td>
<td>34 (46%)</td>
<td>7 (39%)</td>
<td>44 (59%)</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>74</td>
<td>18</td>
<td>74</td>
</tr>
</tbody>
</table>
Table 2: Univariate odds ratio comparing previous history of skin cancer in patients with AKs without follicular extension to patients with AKs with follicular extension.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell</td>
<td>1.18</td>
<td>0.67-2.04</td>
<td>0.57</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cell</td>
<td>1.30</td>
<td>0.72 - 2.27</td>
<td>0.38</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>11.1</td>
<td>1.56-111</td>
<td>0.04</td>
</tr>
<tr>
<td>Overall</td>
<td>1.81</td>
<td>1.01-3.22</td>
<td>0.043</td>
</tr>
</tbody>
</table>
Table 4: Significant variables in a multivariable logistical regression model for predicting the increase in probability of having AK with follicular extension versus AK without follicular extension.

<table>
<thead>
<tr>
<th></th>
<th>Significance</th>
<th>Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.001</td>
<td>1.044</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>0.004</td>
<td>2.51</td>
</tr>
<tr>
<td>Leg</td>
<td>0.012</td>
<td>5.40</td>
</tr>
</tbody>
</table>
Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies.

Lanoue J¹, Chen C¹, Goldenberg G¹.

Abstract
Field cancerization is the process in which a singular cell accumulates genetic mutations following carcinogen exposure and then divides to create a "field" of monoclonal premalignant cells. In this study, microscopically identified actinic keratoses (AKs) were used as markers of field cancerization in all excision specimens of squamous cell carcinomas (SCCs), basal cell carcinomas (BCCs), and malignant melanomas (MMs) received by our institution’s dermatopathology department over a 3- to 6-month period. Our findings provide additional evidence for the theory of field cancerization, its association with cutaneous malignancies, and the need to assess the extent of field damage when determining treatment strategies.
Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies

- $n=149$ excision specimens BCC, SCC & MM

Table 1: Incidence rates of AK by cutaneous malignancy

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th># of Cases Examined (% of Total Cases)</th>
<th># of Cases with AKs Observed (% by Cancer Type)</th>
<th># of Cases with Marginal AKs (% of Previous Column Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC</td>
<td>64 (43%)</td>
<td>21 (33%)</td>
<td>13 (62%)</td>
</tr>
<tr>
<td>SCC</td>
<td>61 (41%)</td>
<td>35 (57%)</td>
<td>20 (57%)</td>
</tr>
<tr>
<td>MM</td>
<td>24 (16%)</td>
<td>6 (33%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>149 (100%)</td>
<td>62 (42%)</td>
<td>35 (56%)</td>
</tr>
</tbody>
</table>

Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies

Table 2: Study population and specimen statistics

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Average Age (Standard Deviation)</th>
<th>% Male (% Female)</th>
<th>Average Specimen Size in cm² (Standard Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC</td>
<td>72 (13)</td>
<td>53% (47%)</td>
<td>2.7 (2.2)</td>
</tr>
<tr>
<td>SCC</td>
<td>63 (13)</td>
<td>57% (43%)</td>
<td>2.1 (2.3)</td>
</tr>
<tr>
<td>MM</td>
<td>75 (13)</td>
<td>58% (42%)</td>
<td>6.2 (5.5)</td>
</tr>
<tr>
<td>Total</td>
<td>68 (14)</td>
<td>56% (44%)</td>
<td>3.0 (3.3)</td>
</tr>
</tbody>
</table>

Table 3: Significant variables in predicting the presence of an AK in the near vicinity of a re-excised cutaneous malignancy as determined by multiple variable regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>Exp (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer type = SCC</td>
<td>P=0.007</td>
<td>2.61</td>
</tr>
<tr>
<td>Cancer location = Head</td>
<td>P=0.044</td>
<td>2.39</td>
</tr>
<tr>
<td>Cancer location = Arm</td>
<td>P=0.042</td>
<td>2.55</td>
</tr>
</tbody>
</table>

Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies

- Of 149 cases, chi-square analysis determined that AKs were observed significantly more often (p=.0125) in SCC re-excisions (57% of 61 SCCs) than BCC (33% of 64 BCCs) or MM (33% of 24 MMs) re-excisions.

- Multivariate regression analysis determined the following variables to be significant in prediction of AKs near malignancies: cancer type of SCC (p=.007) and any type of cutaneous cancer located on the head (p=.044) or on the arms (p=.042).

Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies

Figure 1: Actinic Keratosis and Adjacent Squamous Cell Carcinoma

On the left, an actinic keratosis demonstrates atypical keratinocytes along the basal layer with hyperchromatic nuclei and atypical maturation. On the right, a squamous cell carcinoma is seen which is separated from the AK by a section of histologically normal epithelium.

Figure 2: Actinic Keratosis and Basal Cell Carcinoma

A) Residual basal cell carcinoma B) Actinic keratosis from the same excision with notable parakeratosis and solar elastosis in the dermis.

Figure 3: AK and MM

A) Malignant melanoma B) Incidental actinic keratosis in the same excision specimen; both images exhibit a lymphocytic infiltrate.
Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

Actinic Keratoses

Natural History and Risk of Malignant Transformation in the Veterans Affairs Topical Tretinoin Chemoprevention Trial

Vincent D. Criscione, AB1,2, Martin A. Weinstock, MD, PhD1,2,3, Mark F. Naylor, MD4, Claudia Luque, MD1, Melody J. Elde, MD, MPH5, and Stephen F. Bingham, PhD6, for the Department of Veteran Affairs Topical Tretinoin Chemoprevention Trial Group
Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

- Oklahoma City VA site
- 169 patients
- Mean follow up – 42 months, 7 visits
Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

- 7784 distinct AKs identified during the study
- 411 lesions biopsied
  - 122 (30%) – primary SCC (91 invasive and 31 in situ)
  - 76 (18%) – primary BCC
  - 159 (39%) – AK
  - 16 (4%) – recurrent carcinoma (SCC or BCC)
  - 16 (4%) – seborrheic keratoses
  - 22 – other, nonspecific, or no pathology

Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

• Risk of progression of AK to primary SCC (invasive or in situ)
  – 1 year – 0.60%
  – 4 years – 2.57%

• Risk of progression of AK to primary invasive SCC
  – 1 year – 0.39% (95% CI, 0.26%-0.57%)
  – 4 years – 1.97%

The risk of progression of AK to primary BCC
- 1 year – 0.48%
- 4 years – 1.56%

The risk of progression of AK to any KC (SCC or BCC)
- 1 year – 1.08%
- 4 years – 4.10%

Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

• Baseline AKs had a significantly greater risk of progression to primary SCC (invasive or in situ; P = .02)

• 88/169 patients applied topical tretinoin
  – Rate of malignant transformation did not differ from control group

Actinic Keratosis:
Veterans Affairs Topical Tretinoin Chemoprevention (VATTC) Trial

- 187 primary SCCs on the face or ears (139 invasive and 48 in situ)
  - 65% arose in previously documented AK
- 210 primary BCCs on the face and ears
  - 36% arose in previously documented AK

CONCLUSIONS: In the current study, the authors quantified the malignant potential of clinically diagnosed AKs for both SCC and BCC, although many did not persist, and the results suggested that AKs may play a greater role in the overall burden of keratinocyte carcinomas than previously documented.
Chemoprevention of Basal and Squamous Cell Carcinoma With a Single Course of Fluorouracil, 5%, Cream: A Randomized Clinical Trial

Martin A. Weinstock, MD, PhD; Soe Soe Thwin, PhD; Julia A. Siegel, MD; Kimberly Marcolivio, MEd; Alexander D. Means, MD; Nicholas F. Leader, MS; Fiona M. Shaw, MD; Daniel Hogan, MD; David Eilers, MD; Susan M. Swetter, MD; Suephy C. Chen, MD, MS; Sharon E. Jacob, MD; Erin M. Warshaw, MD, MS; George P. Stricklin, MD, PhD; Robert P. Dellavalle, MD, PhD, MSPH; Navjeet Sidhu-Malik, MD; Nellie Konnikov, MD; Victoria P. Werth, MD; Jonette E. Keri, MD, PhD; Leslie Robinson-Bostom, MD; Robert J. Ringer, PharmD; Robert A. Lew, PhD; Ryan Ferguson, ScD, MPH; John J. DiGiovanna, MD; Grant D. Huang, MPH, PhD; for the Veterans Affairs Keratinocyte Carcinoma Chemoprevention Trial (VAKCC) Group
• **Veterans Affairs Keratinocyte Carcinoma Chemoprevention Trial**
  
  • Randomized, double-blind, placebo-controlled trial
  
  • Topical fluorouracil for chemoprevention of keratinocyte carcinoma
  
  • 12 Veterans Affairs medical centers
  
  • n = 932
  
  – History of at least 2 keratinocyte carcinomas in the past 5 years

• **Fluorouracil 5% (n = 468) vs. vehicle cream (n = 464) to the face and ears twice daily for 2 to 4 weeks**
Results

• Over 4 years:
  – 299 developed a basal cell carcinoma end point (95 in year 1)
  – 108 developed a squamous cell carcinoma end point (25 in year 1)
• No difference between treatment groups in time to first keratinocyte carcinoma
• During the first year
  – 5 participants (1%) in FU group developed SCC vs 20 (4%) in the control group
    • 75% risk reduction ($P = .002$)
  – 11% reduction in BCC risk (45 [10%] in the fluorouracil group vs 50 [11%] in the control group) - not statistically significant

  – Reduction in keratinocyte carcinomas treated with Mohs surgery was observed
“Conclusions and Relevance: A conventional course of fluorouracil to the face and ears substantially reduces surgery for squamous cell carcinoma for 1 year without significantly affecting the corresponding risk for basal cell carcinoma.”
What does TST stand for?

Timed sequential therapy
Imiquimod and Cryotherapy

- Multi-center (US/Canada)
- N= 247 subjects
- 126 Cryo followed by Imiq 3.75%
- 121 Cryo followed by Placebo

- Baseline ≥ 10 AKs on the face
- Cryo some AKs (5-14)
- Leave ≥ 5 AKs for field treatment
- Wait until sufficiently healed (1-2 weeks)
- Average wait time: 12 days
Baseline = 16 AK lesions (protocol requires ≥ 10)

<table>
<thead>
<tr>
<th></th>
<th>Cryo/3.75%</th>
<th>Cryo/Placebo</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>16.1</td>
<td>15.8</td>
<td>16</td>
</tr>
<tr>
<td>Range</td>
<td>(10-39)</td>
<td>(10-50)</td>
<td>(10-50)</td>
</tr>
</tbody>
</table>

7 Lesions Cryo’d (Mean) (protocol requires 5 -14)

9 lesions remain (Mean) (protocol requires ≥ 5)
# Primary Endpoints

## WK 26 (End of Study)

### AKs Treated w/ Cryosurgery

<table>
<thead>
<tr>
<th>Change from Baseline (%) {Week 26/End of Study}</th>
<th>Cryo/Imiq 3.75%</th>
<th>Cryo/Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>-100%</td>
<td>-80.0%</td>
</tr>
<tr>
<td>Mean</td>
<td>-83.9%</td>
<td>-73.1%</td>
</tr>
<tr>
<td>P value</td>
<td>.0008</td>
<td></td>
</tr>
</tbody>
</table>

### ALL AKs*

<table>
<thead>
<tr>
<th>Change from Baseline (%) {Week 26/End of Study}</th>
<th>Cryo/Imiq 3.75%</th>
<th>Cryo/Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>-86.5%</td>
<td>-50.0%</td>
</tr>
<tr>
<td>Mean</td>
<td>-77.4%</td>
<td>-43.3%</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

*All AKs* = AK lesions treated w/ cryosurgery at baseline and AK lesions not treated w/ cryosurgery (baseline, recurrent or new)

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*All AKs = AK lesions treated w/ cryosurgery at baseline and AK lesions not treated w/ cryosurgery (baseline, recurrent or new)*
## Additional Endpoints

**WK 26 (End of Study)**

### AKs Treated w/ Cryosurgery

<table>
<thead>
<tr>
<th></th>
<th>Cryo/Imiq 3.75%</th>
<th>Cryo/Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Patients w/ Complete Clearance</td>
<td>59.5%</td>
<td>29.8%</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

*All AKs* = AK lesions treated w/ cryosurgery at baseline and AK lesions not treated w/ cryosurgery (baseline, recurrent or new)

### All AKs*

<table>
<thead>
<tr>
<th></th>
<th>Cryo/Imiq 3.75%</th>
<th>Cryo/Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Patients w/ Complete Clearance</td>
<td>30.2%</td>
<td>3.3%</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>
An Investigator-initiated Study to Assess the Safety and Efficacy of Imiquimod 3.75% Cream When Used After Cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

GARY GOLDENBERG, MD; RITA V. LINKNER, MD; GISELLE SINGER, BS; AMYLYNNE FRANKEL, MD
Mount Sinai School of Medicine, Department of Dermatology, New York, New York
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

- 20 subjects with at least 3 HAKs on each dorsal hand or forearm underwent cryotherapy treatment to HAKs
- Randomized to have either their right or left dorsal hand or forearm treated with imiquimod 3.75% cream
- **Begin on the same day as cryotherapy**
- 2 wks on, 2 wks off, 2 wks on
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

The number of HAKs in both treatment groups decreased over time with a more pronounced effect observed at weeks 10 and 14 in the cryotherapy/imiquimod group (P < 0.0094).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Timepoint</th>
<th>Number of Subjects</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>P-value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryotherapy plus Imiquimod</td>
<td>20</td>
<td>Baseline</td>
<td>20</td>
<td>6.300000</td>
<td>3.5850567</td>
<td>0.28</td>
<td>2.70</td>
<td>0.0913</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
<td>18</td>
<td>6.1666667</td>
<td>3.8994721</td>
<td>-1.06</td>
<td>3.10</td>
<td>0.9248</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 4</td>
<td>18</td>
<td>5.3888889</td>
<td>3.8369548</td>
<td>-2.94</td>
<td>4.87</td>
<td>0.3816</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>17</td>
<td>3.6470588</td>
<td>2.9987743</td>
<td>-5.00</td>
<td>3.57</td>
<td>0.0169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 10</td>
<td>15</td>
<td>1.8666667</td>
<td>1.3020131</td>
<td>-5.12</td>
<td>3.84</td>
<td>0.0094</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 14</td>
<td>17</td>
<td>1.5294118</td>
<td>1.4627734</td>
<td>-2.13</td>
<td>2.78</td>
<td>-</td>
</tr>
<tr>
<td>Cryotherapy Only</td>
<td>20</td>
<td>Baseline</td>
<td>20</td>
<td>5.5000000</td>
<td>2.5649459</td>
<td>-1.11</td>
<td>1.29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
<td>19</td>
<td>4.3684211</td>
<td>2.4085617</td>
<td>-1.00</td>
<td>2.33</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 4</td>
<td>18</td>
<td>4.5000000</td>
<td>2.4554861</td>
<td>-1.94</td>
<td>2.22</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>17</td>
<td>3.5294118</td>
<td>2.2112679</td>
<td>-2.24</td>
<td>3.19</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 10</td>
<td>16</td>
<td>3.3750000</td>
<td>2.7049338</td>
<td>-2.24</td>
<td>3.19</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 14</td>
<td>17</td>
<td>3.3529412</td>
<td>2.8049326</td>
<td>-2.24</td>
<td>3.19</td>
<td>-</td>
</tr>
</tbody>
</table>

^a P-values were for the comparison, by way of ANOVA with factors of patient and treatment, of mean change from baseline at each week post baseline for the cryotherapy plus imiquimod group compared to the corresponding week in the cryotherapy only group.
Imiquimod 3.75% and cryotherapy in the Treatment of Non-Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Observations</th>
<th>Timepoint</th>
<th>Number of Subjects</th>
<th>Number of Lesions</th>
<th>Change from Baseline in Number of Lesions versus Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>20</td>
<td>7.6000000</td>
<td>Mean 7.6000000, SD 5.3054094</td>
</tr>
<tr>
<td>Cryotherapy plus Imiquimod</td>
<td></td>
<td>Week 2</td>
<td>18</td>
<td>5.5555556</td>
<td>Mean 5.5555556, SD 4.5012707</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 4</td>
<td>17</td>
<td>5.9411765</td>
<td>Mean 5.9411765, SD 4.9177051</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>17</td>
<td>8.0588235</td>
<td>Mean 8.0588235, SD 5.3673852</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 10</td>
<td>15</td>
<td>4.9333333</td>
<td>Mean 4.9333333, SD 4.5113613</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 14</td>
<td>17</td>
<td>2.9411765</td>
<td>Mean 2.9411765, SD 4.9177051</td>
</tr>
<tr>
<td>Cryotherapy Only</td>
<td></td>
<td>Baseline</td>
<td>20</td>
<td>7.6000000</td>
<td>Mean 7.6000000, SD 4.5468323</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
<td>19</td>
<td>5.0526316</td>
<td>Mean 5.0526316, SD 3.3743095</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 4</td>
<td>17</td>
<td>4.4444444</td>
<td>Mean 4.4444444, SD 3.5183924</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>17</td>
<td>4.5294118</td>
<td>Mean 4.5294118, SD 3.0437979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 10</td>
<td>16</td>
<td>3.4375000</td>
<td>Mean 3.4375000, SD 2.3935678</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 14</td>
<td>17</td>
<td>3.2352941</td>
<td>Mean 3.2352941, SD 3.8653818</td>
</tr>
</tbody>
</table>

The number of non-HAKs in the combination therapy group increased at week 6 and then decreased over time. Lesion rates decreased in the cryotherapy alone group.
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

**Incidence of Hypertrophic AKs on the Dorsal Hands and Forearms**

Number of Lesions

- **Baseline**:
  - Cryotherapy + Imiquimod: 6.3
  - Cryotherapy Only: 5.5

- **Week 14**:
  - Cryotherapy + Imiquimod: 3.235
  - Cryotherapy Only: 2.94

*P-value = 0.0094; P-values were for the comparison, by way of ANOVA with factors of patient and treatment, of mean change from baseline at each week post baseline for the cryotherapy + Imiquimod group compared to the corresponding week in the cryotherapy only group.*
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Baseline
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Week 2
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

- Week 6
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

- Week 14/EOS
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Baseline
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Week 2
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Week 4
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

• Week 6
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms

- Week 14/EOS
Imiquimod 3.75% and cryotherapy in the Treatment of Hypertrophic Actinic Keratoses on Dorsal Hands and Forearms
Field treatment with ingenol mebutate gel, 0.015%, 3 weeks after cryosurgery of actinic keratosis is safe and effective

Berman B¹, Swanson N², Goldenberg G³, Hanke W⁴, Tyring S⁵, Werschler W⁶, Knudsen KM⁷, Larsson T⁷

¹University of Miami Miller School of Medicine, Miami, FL, and Center for Clinical and Cosmetic Research, Aventura, FL; ²Oregon Health and Science University, Portland, OR; ³Mount Sinai School of Medicine, New York, NY; ⁴Laser and Skin Surgery Center of Indiana, Carmel, IN; ⁵University of Texas Health Science Center, Houston, TX; ⁶University of Washington School of Medicine, Seattle, WA and Premier Clinical Research, Spokane, WA; ⁷LEO Pharma, Ballerup, Denmark
Study Design

Figure 1. Study Design Diagram

Arm A
- Cryotherapy and Ingenol mebutate, 0.015% gel

Arm B
- Cryotherapy and Vehicle gel
• Complete clearance rates were significantly higher with ingenol mebutate
• Partial clearance (>75%) rates were higher with ingenol mebutate.
• At week 5 mean composite LSR score in the ingenol mebutate group return to a score similar to that of earlier visits
What if you do another 3 day cycle?
• An Investigator-Initiated Study to Assess the Safety and Efficacy of Ingenol Mebutate 0.05% Gel when used after Cryotherapy in the Treatment of Hypertrophic Actinic Keratoses (AK) on Dorsal Hands.
Design

- n=16
- Split hand
- All HT-AK treated with LN2: 2 sprays each 5 seconds with a 5 second interval between
- LN2 not used for any nonHT-AK
- IM 0.05% gel applied same day as LN2
## Results: HT-AK

<table>
<thead>
<tr>
<th>Type</th>
<th>Outcome</th>
<th>Picato</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HT</strong></td>
<td>Baseline # AK's</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.4 (3.14)</td>
<td>4.6 (2.13)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Min, Max</td>
<td>2, 13</td>
<td>1, 10</td>
<td></td>
</tr>
<tr>
<td><strong>Change from Baseline to Day 57 in #AK's</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>-4.6 (2.66)</td>
<td>-2.5 (1.37)</td>
<td></td>
</tr>
<tr>
<td>LS Mean (SE)</td>
<td>-4.3 (0.22)</td>
<td>-2.8 (0.22)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>-4.0</td>
<td>-2.0</td>
<td></td>
</tr>
<tr>
<td>Min, Max</td>
<td>-10, -2</td>
<td>-5, 0</td>
<td></td>
</tr>
<tr>
<td>p-value vs. Control</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Percent Change from Baseline to Day 57</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>-86.34 (16.642)</td>
<td>-51.89 (19.091)</td>
<td></td>
</tr>
<tr>
<td>LS Mean (SE)</td>
<td>-86.1 (4.58)</td>
<td>-52.1 (4.58)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>-100.0</td>
<td>-58.57</td>
<td></td>
</tr>
<tr>
<td>Min, Max</td>
<td>-100.0, -57.1</td>
<td>-75.0, 0.0</td>
<td></td>
</tr>
<tr>
<td>p-value vs. Control</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of subjects CLEAR on Day 57</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>9 (56.3%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of subjects with ≥75% reduction from Baseline to Day 57</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>11 (68.8%)</td>
<td>1 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.0016</td>
<td></td>
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</table>
## Results: nHT-AK

<table>
<thead>
<tr>
<th>Type</th>
<th>Outcome</th>
<th>Picato</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NON-HT</td>
<td>Baseline</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>4.4 (3.24)</td>
<td>3.7 (2.27)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Min, Max</td>
<td>1, 13</td>
<td>1, 9</td>
</tr>
<tr>
<td></td>
<td>Change from Baseline to Day 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>-4.1 (3.38)</td>
<td>0.0 (0.00)</td>
</tr>
<tr>
<td></td>
<td>LS Mean (SE)</td>
<td>-3.8 (0.37)</td>
<td>-0.3 (0.37)</td>
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<tr>
<td></td>
<td>Median</td>
<td>-3.0</td>
<td>0.0</td>
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<td></td>
<td>Min, Max</td>
<td>-13, -1</td>
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<tr>
<td></td>
<td>p-value vs. Control</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>
Efficacy of Cryosurgery and 5-Fluorouracil Cream 0.5% Combination Therapy for the Treatment of Actinic Keratosis

William D. Hoover III, MS; Joseph L. Jorizzo, MD; Adele R. Clark, PA-C; Steven R. Feldman, MD, PhD; Judy Holbrook, LPN, CCRC; Karen E. Huang, MS
Cryosurgery and 5-FU 0.5% Cream

• n=60
• Cryosurgery followed by 5FU 0.5% cream vs vehicle for 1 week
• 28 week follow up
Cryosurgery and 5-FU 0.5% Cream

- Week 8: cryosurgery and 5-FU cream 0.5% more likely to result in complete clearance versus cryosurgery alone
- Week 26: no statistical difference was found in the complete clearance of AK lesions in the treatment group compared to cryosurgery alone
0.5% 5-FU Following Cryotherapy

- Multicenter, randomized, double-blind, vehicle-controlled trial
- 144 patients with ≥5 visible or palpable AKs (face)
- 0.5% 5-FU or vehicle qd x 7 d, residual lesions cryosurgery
- Results at 4 wk
  - Mean AK lesion count reduced by 62.4% with 5-FU vs 28.8% with vehicle (P<.001)
  - Complete clearance: 16.7% with 5-FU vs 0% with vehicle (P<.001)
- Results at 6 mo
  - Mean lesion count reduced by 67% with 5-FU plus cryosurgery vs 45.6% with vehicle plus cryosurgery (P=.01)
  - Complete clearance: 30% with 5-FU plus cryosurgery vs 7.7% with vehicle plus cryosurgery (P<.001)

PDT with Ingenol mebutate vs Ingenol mebutate alone
PDT with Ingenol mebutate vs Ingenol mebutate alone

• **N=24**, randomized, single center
• 4-8 AKs in a discrete facial area (25 cm²)
• **Treatment groups**: (N=8 per group)
  1. ALA-PDT x 2 (4 weeks apart)
  2. ALA-PDT → IM 0.015% for 3 days (2 weeks apart)
  3. IM x 1 (0.015% for 3 days)*
• **ALA-PDT protocol**
  – 20% ALA solution, 1-hour incubation, double-coat applied to 25 cm² area
  – Blue light: 16:40
• AKs counted at baseline and study end (day 57 or day 71)

*treatment applied in-office
All treatment groups had statistically significant AK lesion reductions compared to baseline

- The ALA-PDT x 2 group had the greatest numerical reduction, but this was not statistically significant vs. the sequential ALA-PDT group or the IM alone group (p=0.90, p+0.136 respectively)

AK Lesion Reductions at Study End
(*Day 57, **Day 71)

<table>
<thead>
<tr>
<th></th>
<th>ALA-PDT x 2</th>
<th>ALA-PDT → IM</th>
<th>IM x 1</th>
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</thead>
<tbody>
<tr>
<td>Baseline AK #*</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>AK Lesion Reductions</td>
<td>97.5%**</td>
<td>86.7%**</td>
<td>91.7%*</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.00001</td>
<td>p&lt;0.00001</td>
<td>p&lt;0.00001</td>
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</tbody>
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*REPRESENTATIVE PATIENT in each group
• Composite LSR score was calculated from the sum of the individual LSR categories, with a maximum severity score of 24

<table>
<thead>
<tr>
<th>Peak Composite LSR Score</th>
<th>ALA-PDT × 2</th>
<th>ALA-PDT → IM</th>
<th>IM × 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSR score</td>
<td>4.625</td>
<td>10.375</td>
<td>12.625</td>
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<tr>
<td>p-value</td>
<td>p=0.0011</td>
<td>p=0.0004</td>
<td></td>
</tr>
</tbody>
</table>

Day 0: **4 AKs present**, LSR= 2

Day 4, LSR=2

Day 14, LSR=1

Day 29: day of 2\textsuperscript{nd} PDT, **2 AKs present**, LSR=2

Day 43: 14 days post 2\textsuperscript{nd} PDT, LSR=7

Day 71: 42 days post 2\textsuperscript{nd} PDT – **Zero AKs**, LSR=0
Day 0: 5 AKs present, LSR= 2
Day 14: 2 AKs present, LSR= 1
Day 16: Day 2 post IM, LSR= 8
Day 18: Day 4 post IM, LSR= 4
Day 22: Day 8 post IM, LSR= 4
Day 29: Day 15 post IM, LSR= 1, Zero AKs
Day 0: 6 AKs present, LSR= 2

Day 2: LSR= 7

Day 4: LSR= 9

Day 15: LSR= 3

Day 29: LSR= 2

Day 29: 1 AK present, LSR= 1

IM x 1
SEQUENTIAL TREATMENT:
ALA-PDT → Imiquimod

Treatment of Actinic Keratoses With Sequential Use of Photodynamic Therapy and Imiquimod 5% Cream

Michael Shaffelburg MD FR.CPC
Valley Professional Center, Kentville, Nova Scotia, Canada
PDT followed by imiquimod 5%

• Randomized, vehicle-controlled, split-face study
• N=25
• Adults with ≥ 10 facial AKs
• ALA-PDT Protocol
  – Baseline & Month 1: ALA-PDT
    • Acetone scrub → microdermabrasion → 20% ALA solution broad area → 1-hour incubation → blue light x 8 minutes
  – Month 2: Imiquimod, split-face (vehicle on other side)
    • 2 times per week x 16 weeks

Results: 6 months

Reduction in AK Lesion Count (%, median)

Results: 12 months

**Safety**

- Common: erythema, flaking/scaling, scabbing/crusting
- Erythema: 87.5% (N=21) during IMQ treatment
- Local skin reaction rest periods: 1 week (N=1), 2 weeks (N=2)
- No discontinuations
- No systemic AEs, including flu-like AE’s

### AK Lesion Reduction

<table>
<thead>
<tr>
<th></th>
<th>ALA-PDT → IMQ</th>
<th>ALA-PDT → Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline AK (median)</td>
<td>23.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Month 12</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>AK Lesion Reduction (mean)</td>
<td>89.9% (p=0.0023)</td>
<td>74.5%</td>
</tr>
<tr>
<td>Complete Clearance</td>
<td>N=2</td>
<td>N=2</td>
</tr>
</tbody>
</table>

BF-200 10% ALA Gel
Photodynamic Therapy
Stabilization of ALA by binding to the outside of the nanovesicles

Electrostatic Interaction between ALA with Lecithin

Lecithin (Phosphatidylcholine)

Interaction

5-aminolevulinic acid

BF-200 ALA Gel

MAL Cream

- Penetration studies in a porcine ex-vivo skin model
- Nanoemulsion BF-200 optimizes transport of 5-ALA through the Stratum Corneum
- Significantly deeper PpIX induction with this formulation (down to basal membrane)
- No PpIX induction below the basal membrane layer

Maisch & Szeimies, Study report, 2008
**Red Light Source (BF-RhodoLED)**

Red Light Increases the Depth of Penetration
Complete Patient Clearance – per US PI Data for PDT Drugs

Thank You