
**eMethods.** Statistical Assumption to Determine the Sample Size of 36

**eFigure.** Redness Grading Scale

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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Statistical Assumption to Determine the Sample Size of 36

In this study, we determined the sample size to obtain statistical significance in terms of both safety and efficacy. First, we considered the occurrence rate of significant adverse event (AE) for placebo group as 5%, and considered the non-admissible occurrence rate of significant AE for active drug group as > 25%. From the viewpoint of safety, we decided to carry out dose-escalation if the observed occurrence rate of significant AE is less than 25%, otherwise we decided not to increase the dose level. Then, we calculated probabilities that the dose level is not wrongly increased when the true occurrence rate of significant AE is 5%. The results were shown in the following table.

<table>
<thead>
<tr>
<th>Number</th>
<th>No. of patients experiencing AEs requiring withdrawal</th>
<th>Incidence of AEs requiring withdrawal</th>
<th>Probability of withdrawal of the active drug in error with a 5% true occurrence rate</th>
<th>Probability of correct withdrawal of the active drug with a 25% true occurrence rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2</td>
<td>0.400</td>
<td>0.023</td>
<td>0.367</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.333</td>
<td>0.033</td>
<td>0.466</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0.286</td>
<td>0.044</td>
<td>0.555</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0.250</td>
<td>0.057</td>
<td>0.633</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0.333</td>
<td>0.008</td>
<td>0.399</td>
</tr>
</tbody>
</table>

From the above results, we considered 7 or 8 subjects as appropriate sample size in terms of safety.

Next, we considered a sample size in terms of efficacy. The previous study showed that the mean and standard deviation (SD) of the efficacy evaluation score (same endpoint as this study) in the sirolimus group (n=7) and placebo group (n=7) were 3.10±0.98 and 0.10±0.13, respectively. The calculation of the effect size was performed by using the SD of placebo group and using double confidence limit method (Uesaka, 2003) with 70% or 80% one-sided confidence interval conservatively. The effect size and required sample size were indicated in the following table when we assumed Student-t test.

<table>
<thead>
<tr>
<th>Confidential coefficient</th>
<th>70%</th>
<th>80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limit of sirolimus</td>
<td>2.89</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>Upper limit for placebo</td>
<td>Difference of means</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the results above, we found 7 subjects needed with alpha = 5% and power = 90%. However, the final analysis for the primary endpoint is Wilcoxon rank sum test. So, we needed $7 \times 1.05 = 8$ subject per group. In addition to this result, we must consider adult group and pediatric group. We assumed there was no difference between adult and pediatric group for efficacy. Furthermore, the number of patients assigned to be placebo group should be as few as possible. Therefore, we calculated statistical power under some combinations of patients when we compared active drug group with placebo group by pooling adult and pediatric patients of each group. The results were as follows.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>A</th>
<th>power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Pediatric</td>
<td>(active drug, placebo)</td>
</tr>
<tr>
<td>(active drug, placebo)</td>
<td>(active drug, placebo)</td>
<td>(active drug, placebo)</td>
</tr>
<tr>
<td>(4, 1)</td>
<td>(3, 1)</td>
<td>(7, 2)</td>
</tr>
<tr>
<td>(4, 2)</td>
<td>(3, 2)</td>
<td>(7, 4)</td>
</tr>
<tr>
<td>(4, 1)</td>
<td>(4, 2)</td>
<td>(8, 2)</td>
</tr>
<tr>
<td>(4, 2)</td>
<td>(4, 2)</td>
<td>(8, 4)</td>
</tr>
</tbody>
</table>

A: Pooled data from adult and pediatric patients.

From the results, the last combination was considered to be better. Therefore, in terms of both safety and efficacy, the sample size was 12 per dose, totally 36 patients in this study.
Six stages of redness using a PANTONE COLOR BRIDGE Coated (PANTONE) set were indicated. A score of 1 is shown by the color 489c or a lighter color, and a score of 6 is represented by a red color more intense than 704 C. Improvement scores of 2.0, 1.0, 0.5, 0 and -1 represented a change in the evaluation values of 9 or more, 6 to 8, 3 to 5, less than 2 to -2, and -3 to -12, respectively. The evaluation value was calculated as follows: (sum of the scores of 3 target tumors before treatment) - (sum of the scores of 3 target tumors after treatment). As the change of redness became pale, the evaluation value in Figure 3 is shown as a minus volume. Improvement score of redness is used to calculate improvement factor which is represented as the sum of improvement score of redness and the score of tumor volume reduction.
### eTable 1. Baseline Clinical Characteristics of Patients

#### A. Child group

<table>
<thead>
<tr>
<th>Concentration of sirolimus</th>
<th>Placebo</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.2%</th>
<th>Active drug group (Sum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Gender Man</td>
<td>3 (50%)</td>
<td>0</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Woman</td>
<td>3 (50%)</td>
<td>4 (100%)</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Age Average</td>
<td>9.0</td>
<td>11.0</td>
<td>12.5</td>
<td>10.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Oldest/youngest</td>
<td>13 / 6</td>
<td>18 / 6</td>
<td>17 / 8</td>
<td>17 / 6</td>
<td>18 / 6</td>
</tr>
<tr>
<td>Sum of the tumor volume of 3 target tumors (mm³)</td>
<td>Average</td>
<td>29 ± 23</td>
<td>103 ± 109</td>
<td>59 ± 46</td>
<td>26 ± 10</td>
</tr>
<tr>
<td>Max/Min volume</td>
<td>68 / 9</td>
<td>257 / 16</td>
<td>120 / 20</td>
<td>40 / 16</td>
<td>257 / 16</td>
</tr>
<tr>
<td>The average degree of the color of 3 target tumors</td>
<td>Average</td>
<td>2.7</td>
<td>2.6</td>
<td>3.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Maxi/Min</td>
<td>7 / 0</td>
<td>4 / 0</td>
<td>8 / 0</td>
<td>4 / 0</td>
<td>8 / 0</td>
</tr>
<tr>
<td>Number (ratio) of patients with complications</td>
<td>6 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
<td>3 (75%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Number (ratio) of patients with mental retardation</td>
<td>2 (33%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Number (ratio) of patients with epilepsy</td>
<td>6 (100%)</td>
<td>4 (100%)</td>
<td>3 (75%)</td>
<td>3 (75%)</td>
<td>10 (83%)</td>
</tr>
</tbody>
</table>

SD: Standard deviation

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### B. Adult group

<table>
<thead>
<tr>
<th>Concentration of sirolimus</th>
<th>Placebo</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.2%</th>
<th>Active drug group (Sum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Gender</td>
<td>Man</td>
<td>3 (50%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Woman</td>
<td>3 (50%)</td>
<td>2 (50%)</td>
<td>3 (75%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Age</td>
<td>Average</td>
<td>29.0</td>
<td>30.8</td>
<td>27.5</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>Oldest/youngest</td>
<td>42 / 19</td>
<td>47 / 20</td>
<td>42 / 19</td>
<td>37 / 19</td>
</tr>
<tr>
<td>Sum of the tumor volume of 3 target tumors (mm$^3$)</td>
<td>Average</td>
<td>105 ± 90</td>
<td>49 ± 20</td>
<td>108 ± 139</td>
<td>94 ± 67</td>
</tr>
<tr>
<td></td>
<td>Max/Mini</td>
<td>68 / 9</td>
<td>257 / 16</td>
<td>120 / 20</td>
<td>40 / 16</td>
</tr>
<tr>
<td>The average degree of the color of 3 target tumors</td>
<td>Average</td>
<td>3.3</td>
<td>2.8</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Max/Mini</td>
<td>7 / 0</td>
<td>4 / 0</td>
<td>8 / 0</td>
<td>4 / 0</td>
</tr>
<tr>
<td>Number (ratio) of patients with complications</td>
<td>6 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Number (ratio) of patients with mental retardation</td>
<td>3 (50%)</td>
<td>1 (25%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Number (ratio) of patients with epilepsy</td>
<td>3 (50%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>6 (50%)</td>
</tr>
</tbody>
</table>

SD: Standard deviation
eTable 2. Actual Value of General Improvement (Patient Number on Each Improvement Stage)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sirolimus concentration</th>
<th>Patient numbers</th>
<th>Stage of general improvement</th>
<th>Median (IQR)</th>
<th>( P ) Value for Wilcoxon Rank Sum Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1  2  3  4  5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>placebo</td>
<td>12</td>
<td>1  1  1  9  0</td>
<td>4.00 (3.50-4.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>8</td>
<td>2  2  3  1  0</td>
<td>2.50 (1.50-3.00)</td>
<td>.008</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>8</td>
<td>2  1  2  3  0</td>
<td>3.00 (1.50-4.00)</td>
<td>.080</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>8</td>
<td>1  6  1  0  0</td>
<td>2.00 (2.00-2.00)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Sum of the active drug</td>
<td>24</td>
<td>5  9  6  4  0</td>
<td>2.00 (2.00-3.00)</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>placebo</td>
<td>6</td>
<td>1  0  0  5  0</td>
<td>4.00 (4.00-4.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>4</td>
<td>2  0  2  0  0</td>
<td>2.00 (1.00-3.00)</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>4</td>
<td>2  0  1  1  0</td>
<td>2.00 (1.00-3.50)</td>
<td>.119</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>4</td>
<td>1  3  0  0  0</td>
<td>2.00 (1.50-2.00)</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>Sum of the active drug</td>
<td>12</td>
<td>5  3  3  1  0</td>
<td>2.00 (1.00-3.00)</td>
<td>.014</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult group</td>
<td>placebo</td>
<td>0.05%</td>
<td>0.1%</td>
<td>0.2%</td>
<td>Sum of the active drug group</td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>6 0 1 1 4 0</td>
<td>4 0 2 1 1 0</td>
<td>4 0 1 1 2 0</td>
<td>4 0 3 1 0 0</td>
<td>12 0 6 3 3 0</td>
</tr>
<tr>
<td></td>
<td>4.00 (3.00-4.00)</td>
<td>2.50 (2.00-3.50)</td>
<td>3.50 (2.50-4.00)</td>
<td>2.00 (2.00-2.50)</td>
<td>2.50 (2.00-3.50)</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.190</td>
<td>0.476</td>
<td>0.043</td>
<td>0.084</td>
</tr>
</tbody>
</table>

1: remarkable improvement, 2: moderate improvement, 3: slight improvement, 4: unchanged. 5: worse
eTable 3. Actual Value of Patient Satisfaction (Patient Number on Each Satisfaction Stage)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sirolimus concentration</th>
<th>Patient numbers</th>
<th>Grade of patient satisfaction</th>
<th>Median (IQR)</th>
<th>P Value for Wilcoxon Rank Sum Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>placebo</td>
<td>12</td>
<td>2 0 3 5 2</td>
<td>4.00 (3.00-4.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>8</td>
<td>2 1 5 0 0</td>
<td>3.00 (1.50-3.00)</td>
<td>.021</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>8</td>
<td>3 0 2 3 0</td>
<td>3.00 (1.00-4.00)</td>
<td>.143</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>8</td>
<td>0 5 2 0 1</td>
<td>2.00 (2.00-3.00)</td>
<td>.059</td>
</tr>
<tr>
<td>Sum of the active drug group</td>
<td>placebo</td>
<td>24</td>
<td>5 6 9 3 1</td>
<td>3.00 (2.00-3.00)</td>
<td>.016</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>6</td>
<td>1 0 0 4 1</td>
<td>4.00 (4.00-4.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>4</td>
<td>1 1 2 0 0</td>
<td>2.50 (1.50-3.00)</td>
<td>.043</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>4</td>
<td>2 0 2 0 0</td>
<td>2.00 (1.00-3.00)</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>4</td>
<td>0 3 1 0 0</td>
<td>2.00 (2.00-2.50)</td>
<td>.024</td>
</tr>
<tr>
<td>Sum of the active drug group</td>
<td>placebo</td>
<td>12</td>
<td>3 4 5 0 0</td>
<td>2.00 (1.50-3.00)</td>
<td>.030</td>
</tr>
<tr>
<td>Adult group</td>
<td>placebo</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.05%</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>0.1%</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>0.2%</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sum of the active drug group</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

eTable 4. Number and Ratio of Patients With Dry and Irritated Skin

A. Dry skin

<table>
<thead>
<tr>
<th>Group of all participants (number)</th>
<th>Concentration of sirolimus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (12)</td>
</tr>
<tr>
<td>All patients (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>Child group (18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Adult group (18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (16.7%)</td>
</tr>
</tbody>
</table>

Dry skin: dryness without sign of inflammation.

B. Irritated skin

<table>
<thead>
<tr>
<th>Group of all participants (number)</th>
<th>Concentration of sirolimus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (12)</td>
</tr>
<tr>
<td>All patients (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td>Child group (18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Adult group (18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (33.4%)</td>
</tr>
</tbody>
</table>

Irritated skin, i.e., skin with signs of inflammation
eTable 5. Ratio of Patients With Positive Blood Sirolimus Concentration at Every Visit

<table>
<thead>
<tr>
<th>Examination point</th>
<th>0 day</th>
<th>2 W</th>
<th>4 W</th>
<th>8 W</th>
<th>12 W</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>1 hour after treatment</td>
<td>Through</td>
<td>Through</td>
<td>Through</td>
</tr>
<tr>
<td>Adult</td>
<td>0.05%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>0</td>
<td>25 (0.15)</td>
<td>25 (0.17)</td>
<td>50 (0.12)</td>
</tr>
<tr>
<td>Child</td>
<td>0.05%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25 (0.14)</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>0</td>
<td>25 (0.16)</td>
<td>75 (0.20)</td>
<td>100 (0.20)</td>
</tr>
</tbody>
</table>

The number in parentheses indicates the highest rapamycin concentration (ng/ml)