Four-week-old C57BL/10ScSn-Dmdmdx/J (BL/10) mdx mice (N=60) were treated with Elamipretide (MTP-131; ELAM) is a clinical stage mitochondrial-targeting peptide. Mitochondria are postulated to modulate PMO efficacy by mediating cell injury.

For the comparison of healthy mouse (BL/10 mouse) versus saline (mdx mouse), an unpaired t-test was performed and mean±standard error of the mean (SEM) was calculated.

Variables measured included muscle force (extensor digitorum longus [EDL] max and specific force).

At the end of the 7-week study duration:
- Dystrophin expression in the muscle was quantified via western blot and immunofluorescence
- Percent inflammation (hematoxylin and eosin [H&E] staining; TA)
- In-vitro lation development from EDL muscles

For the comparison of healthy mouse (BL/10 mouse) versus saline (mdx mouse), an unpaired t-test was performed and mean±standard error of the mean (SEM) was calculated.

For the comparison of the saline and treatment groups (PMO, ELAM, and combination therapy [PMO+ELAM]), an ordinary one-way ANOVA followed by Dunnett’s Multiple Comparison test was performed and mean±SEM was calculated.

Table 1. Average Dystrophin Protein Levels for the Four Treatment Groups: Saline, PMO, ELAM and Combination

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Range of Dystrophin Protein Levels (Min, Max)</th>
<th>Average Dystrophin Protein Level</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>(0.0%, 1.2%)</td>
<td>0.7%</td>
<td>0.4%</td>
</tr>
<tr>
<td>PMO</td>
<td>(1.2%, 7.4%)</td>
<td>3.7%</td>
<td>2.1%</td>
</tr>
<tr>
<td>ELAM</td>
<td>(0.0%, 1.2%)</td>
<td>0.7%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Combination (PMO+ELAM)</td>
<td>(2.1%, 20.8%)</td>
<td>7.9%</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

Combination therapy (PMO+ELAM) more than doubled the mean level of dystrophin protein (7.9% ± 6.0%, P<0.05 versus PMO alone) (Figure 3)

Figure 2. Study Design

RETROSPECTIVE PRECLINICAL STUDY TO EXAMINE IF ELAMIPRETE POTENTIATES DYSTROPHIN EXPRESSION

Immunofluorescence staining with an anti-dystrophin antibody provided corroborating support for the increase in dystrophin expression (Figure 4).

Figure 3. Mean Levels of Dystrophin Protein for the Four Treatment Groups: Saline, PMO, ELAM and Combination

Figure 4. Immunofluorescence TA Percent Dystrophin and Dystrophin Expression: Immunofluorescence Staining

Figure 5. Percent Inflammation in TA Tissues

CONCLUSIONS

This preclinical study provides compelling data that elamipretide combined with an exon-skipping PMO augments dystrophin expression in mdx mice.

Combination therapy (PMO+ELAM) more than doubled the mean level of dystrophin protein.

Elamipretide alone trended to lower markers of inflammation by 34% on average (Figure 5).

Inflammation was significantly higher in the mdx mouse muscle.

Elamipretide alone trended to lower markers of inflammation by 34% on average (Figure 5).

In vitro specific force production was significantly lower in the extensor digitorum longus (EDL) muscle from mdx mice, with no treatment effects observed.

REFERENCES: