dNerva® System Overview
TLD Mechanism of Action and Preclinical Evidence

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Targeted Lung Denervation (TLD)

**Denervation**
- Interrupts vagus nerve signaling to and from the lung (sensory disruption and decreased acetylcholine release)

**Lung**
- Attenuates basal parasympathetic tone
- Decrease airway smooth muscle tone
- Decrease mucous secretion
- Blunts pulmonary nerve reflexes
- Decrease airway hyper-responsiveness and decreases exacerbations

**Targeted**
- Anatomically to only the lung
- To the depth of the pulmonary nerves
dNerva® Lung Denervation System

- **Radio-Frequency Generator** for ablative therapy
- **Thermoelectric Plate** to cool fluid to protect airway wall
- **Prompt Screen** to control console and guide user
- **Fluid Pump** to circulate chilled fluid through catheter
- **Dual-Cooled Catheter**
Chilled fluid from the console flows through the electrode and balloon (indicated by the blue arrows)

- Inflates the balloon and provides *consistent contact* of the electrode with the airway wall
- Cools the inner surface of the airway to protect it while focusing heating effect to the depth of the pulmonary nerves
Cooling Protects Airway Wall while Targeting at Depth
TLD Procedure: Four steps / Four positions

- Four steps in the procedure
- Four positions to achieve circumferential treatment
- General anesthesia used
- Fluoroscopy used

1. Inflate
2. Confirm
3. Activate
4. Repeat
TLD disruption of vagus nerve signaling is a logical therapeutic target in COPD management

Pulmonary vagal nerves (vagal afferent and parasympathetic efferent) are mediators of COPD pathophysiology

- Airflow limitation and gas trapping
- Increased basal parasympathetic smooth muscle tone
- Mucus hypersecretion
- Innervate and stimulate submucosal gland hypersecretion
- **COPD Exacerbations (AECOPD)**
  - Neuro-hyperresponsiveness contributes to airway hyperresponsiveness and increased symptom burden during exacerbation

2. Colebatch et al J Appl Physiol 1963
Vagal nerve meditates tonic control of airway smooth muscle

Electrical stimulation of the vagus nerve produces immediate increases in airway resistance.

Bilateral cervical vagotomy produced reduced inspiratory resistance to airflow.

Vagal nerve mediates tonic control of smooth muscle in the lungs

*indicated $p < 0.05$ vs intact vagal control

**TABLE 1. Effect of vagotomy on lung mechanics**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Animals</th>
<th>Lung compliance, ml/cm H$_2$O kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td>3.53±2.05</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>2.16±.77*</td>
</tr>
<tr>
<td>Inf</td>
<td>13</td>
<td>3.71±2.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tidal volume, ml/m$^2$ BSA, BTPS</th>
<th>Conditions</th>
<th>No. of Animals</th>
<th>286±58</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td></td>
<td>286±58</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td></td>
<td>429±91</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of breaths/min</th>
<th>Conditions</th>
<th>No. of Animals</th>
<th>43.8±13.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td></td>
<td>43.8±13.8</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td></td>
<td>24.6±7.5*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ventilation, liters/min m$^2$ BSA, BTPS</th>
<th>Conditions</th>
<th>No. of Animals</th>
<th>12.2±3.43</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td></td>
<td>12.2±3.43</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td></td>
<td>10.0±2.87*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insp. resist. to airflow, cm H$_2$O/liter sec</th>
<th>Conditions</th>
<th>No. of Animals</th>
<th>1.23±.22</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td></td>
<td>1.23±.22</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td></td>
<td>.96±.24*</td>
</tr>
</tbody>
</table>
Vagal nerve modulates mucus hypersecretion

Submucosal glands densely innervated by parasympathetic system\(^1\)

Stimulation of parasympathetic efferents increase secretion rates to \(\sim 2\)x the basal rate\(^2\)

Sensory axons (C fibers and stretch receptors) stimulate mucus secretion via central reflexes\(^3,4,5\)

Glands from chronic bronchitis, COPD and cystic fibrosis patients exaggerated response to nerve input\(^6\)

1. Cuthbert et al Physiological Reports 2015
5. Davis et al J Appl Physiol 1982
6. Sturgess et al Exper and Mol Path 1972
Lung Nerve structure/function: Reflex bronchoconstriction

- Peripheral nerve neurons
  - Soma or cell body
    - Sensory neuron: ganglia near CNS
    - Motor neuron: within CNS
  - Axon
    - Projects from soma to target

- Sensory neuron, vagal afferent
- Motor neuron, parasympathetic efferent
- Parasympathetic Ganglia
- Sensory Afferent Action potential (AP)
- Motor Efferent Action potential (AP)

1) Acute stimuli
2) Sensory AP
3) Motor AP
4) Activation of Pulmonary ganglion
5) ACh release: Bronchoconstriction Mucus Hypersecretion

Brain Stem
- Dorsal motor nucleus (CN X)
- Nucleus ambiguus
- Nucleus of solitary tract
- Jugular and Nodose ganglia

Lung Nerve structure/function: Reflex bronchoconstriction

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Reflex bronchoconstriction mediated by Vagus nerve

Vagotomy abolishes reflex bronchoconstriction
  ◦ Evolutionarily conserved reflex across multiple species

Vagal nerve essential to bronchial hyperresponsiveness

- Vagotony reverses hyperresponsiveness associated with chronic/acute inflammation\(^1\)

- Vagotony reverses hyperresponsiveness associated with viral infection\(^2\)

- Hyperactive sensory fibers in inflammatory environment contribute to increased symptoms\(^3,4\)

2. Buckner et al Am rev Respir Dis 1985
Airway hyper-responsiveness and Exacerbations

“Airway hyper-responsiveness can exist without diagnosis of asthma and has been shown to be an independent predictor of COPD and respiratory mortality in population studies....”

-- GOLD 2018

Airway hyper-responsiveness in COPD patients

- Associated with long-term mortality in COPD (p < 0.05)¹
- Correlated to worsening symptom score (r = 0.87)²
- Correlated to prior year exacerbations (r = 0.77)²
- Associated with air trapping (p = 0.021)³
- Associated with lung inflammation (p < 0.05)³,⁴

Majority of AECOPD are associated with a viral or bacterial infection⁵,⁶,⁷

- Transient hyper-responsiveness a feature of pulmonary infection

2. Zanini et al Int J COPD 2015
5. Seemungal et al AJRCCM 2001
7. Sethi et al NEJM 2002
TLD disrupts airway hyperresponsiveness

• Peripheral nerve neurons
  • Soma or cell body
    • Sensory neuron: ganglia near CNS
    • Motor neuron: within CNS
  • Axon
    • Projects from soma to target

1) Acute stimuli
2) Sensory reflex disrupted
3) Exaggerated Motor AP
4) No activation of Pulmonary ganglion
5) ACh release: Prevented

TLD treatment site: die back of nerve fibers

• Sensory neuron, vagal afferent
• Motor neuron, parasympathetic efferent
• Parasympathetic Ganglia
• Sensory Afferent Action potential (AP)
• Motor Efferent Action potential (AP)
## Nuvaira Pre-clinical Bench / Radiography / CT Studies

<table>
<thead>
<tr>
<th>#</th>
<th>Study</th>
<th>N</th>
<th>Model</th>
<th>Key Findings</th>
</tr>
</thead>
</table>
| 1  | Bench Tissue Testing                           | >200  | Animal tissue      | • Characterization of catheter and system  
   • Varied power, treatment time, coolant temperature (how is this a “finding”? )                                                                                 |
| 2  | Finite Element Modeling                        | 12    | Computer           | • Demonstration of voltage, current and thermal profiles  
   • Confirmation of bench testing and animal study findings                                                                                                         |
| 3  | Animal Studies¹                                | 184   | Sheep/Dog          | • TLD disrupts bronchial nerve branches, alters sensory signals, improves airway resistance  
   • Effect of TLD sustained for almost 2 years  
   • Surface cooling with balloon protects airway surface and prevents stenosis                                                                                   |
| 3  | Human Airway and Surrounding Anatomy – non COPD| 75    | Human Chest CT Scans | • Cataloged human airway dimensions and geometry  
   • Cataloged relative location of esophagus                                                                                                                      |
| 4  | Human Airway and Surrounding Anatomy in Stage 4 COPD | 20    | Human Chest CT Scans | • Cataloged human airway dimensions and geometry  
   • Cataloged relative location of esophagus                                                                                                                      |
| 5  | Human Airway Nerve Anatomy                     | 6     | Human Cadaver      | • Nerves along main bronchi  
   • Posterior predominance  
   • 90% of nerves within 5mm of airway surface (data under manuscript development)  
   • Similar structural layout as sheep                                                                                                                              |
| 6  | Human Cadaver                                  | 15/6  | Human Cadaver      | • 20/15 W ablates nerve axons                                                                                                                                          |

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1. Hummel et al J Appl Physiol 2018

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Pulmonary vagal nerves (vagal afferent and parasympathetic efferent) are mediators of COPD pathophysiology

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