LIQ865A is a slow release microparticle formulation of bupivacaine, is well-tolerated and does not interfere with wound healing after subcutaneous dosing in rats and minipigs.

### Introduction and Objective

LIQ865A is a bupivacaine (Bup) formulation developed by Liquidia Technologies, Inc. for the management of local post-operative pain using a proprietary process technology called PRINT® (Particle Replication In Non-wetting Templates). LIQ865A is 25 µm hexagonal particle comprised of approximately 55% bupivacaine and 45% poly(lactic-co-glycolic acid) (PLGA). Particles are suspended in a custom vehicle for subcutaneous (SC) administration. LIQ865A is designed to slowly release bupivacaine at the surgical site over 3 to 5 days providing a longer pain management solution as compared to the current state of the art while reducing the potential for systemic toxicity secondary to an increase in bupivacaine plasma concentrations.

To support clinical testing, pivotal GLP studies were conducted in both Sprague Dawley rats and Yorkshire miniswine to assess local tolerability and the potential impact of LIQ865A on wound healing.

### STUDY DESIGN and METHODS

#### Single SC Administration Toxicity Study In Rats

The core process involves four basic steps that produces a bulk powder consisting of particles of uniform composition.

1. Create a film of the desired composition on a delivery sheet.
2. Fills the mold cavities.
3. Remove particles from the mold template.
4. Forms the final product in a desired shape and size using a molding process leveraged to produce a wide range of shapes, sizes, and composition.

### PRINT® Fabrication Process

During PRINT manufacturing, components are formed into the desired shape and size using a molding process that produces a bulk powder consisting of particles of uniform size, shape, and composition. Several variables can be leveraged to produce a wide range of shapes, sizes, and composition.

The core process involves four basic steps:

1. Create a film of the desired composition on a delivery sheet.
2. Laminate the film with a mold template where the material fills the mold cavities.
3. Remove particles from the mold template.
4. Collect particles to create a particle suspension or dry powder.

### STABILITY TESTING

To support clinical testing, pivotal GLP studies were conducted in both Sprague Dawley rats and Yorkshire miniswine to assess local tolerability and the potential impact of LIQ865A on wound healing.

#### STUDY DESIGN and METHODS

**Regulatory Compliance:**

- **GLP:**
  - Animals: Sprague Dawley rats, 6 weeks of age at Study Day 1
  - In-life data: mortality, clinical signs, body weights, food consumption, clinical pathologies
  - Dosing Site Observations:
    - Day 1-14, daily after that for animals that showed continued injection site signs
  - Blood Collection Time Points:
    - Blood Collection: direct venipuncture of jugular vein (5-6 blood samples per rat)
    - No. of animals (satellite groups): 3/sex/time point
  - Toxicokinetics:
    - Assay: Liquid chromatography (LC) using liquid chromatography/mass spectrometry (LC-MS/MS) method
  - Bioanalytical Method: Validated LC-MS/MS assay (AIT Bioscience)
  - Following dosing, incision was sutured closed and bandaged
  - Incision sites will be monitored for signs of infection (erythema, edema, accumulation of fluid, scabbing)

**Post-mortem data:**

- Gross pathology, organ weights, histopathology (dosing site tissues including skin, subcutis, underlying muscle and inguinal lymph nodes)

**Pathology:**

- Hemorrhage, fibrin, inflammation
  - Increased fibrosis/healing along incision line
  - Inflammatory response and fibrosis localized
  - Vascularized fibrous connective tissue, macrophages, giant cells, fibroblasts
  - No giant cells observed in low dose group.
  - Macrophages and giant cells were not present.

**Single SC Administration Toxicity Study in Minipigs Full-Thickness Incisional Model**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bup (mg/kg)</th>
<th>Particle Conc. (mg/mL)</th>
<th>No. of Animals/Incision/Batch</th>
<th>Incision Site/Pathology</th>
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<td>Controls and sham</td>
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<tr>
<td>LIQ865A</td>
<td>6</td>
<td>6.0</td>
<td>6</td>
<td>Controls and sham</td>
</tr>
</tbody>
</table>

#### Toxicokinetics

- Assay: Liquid chromatography (LC) using liquid chromatography/mass spectrometry (LC-MS/MS) method
- Bioanalytical Method: Validated LC-MS/MS assay (AIT Bioscience)
- Following dosing, incision was sutured closed and bandaged
- Incision sites will be monitored for signs of infection (erythema, edema, accumulation of fluid, scabbing)

**Regulatory Compliance:**

- **GLP:**
  - Animals: Yorkshire miniswine, ~9 weeks of age at Study Day 1
  - In-life data: mortality, clinical signs, body weights, food consumption, clinical pathologies
  - Dosing Site Observations:
    - Day 1-14, daily after that for animals that showed continued injection site signs
  - Blood Collection Time Points:
    - Blood Collection: direct venipuncture of jugular vein (5-6 blood samples per rat)
    - No. of animals (satellite groups): 3/sex/time point
  - Toxicokinetics:
    - Assay: Liquid chromatography (LC) using liquid chromatography/mass spectrometry (LC-MS/MS) method
    - Bioanalytical Method: Validated LC-MS/MS assay (AIT Bioscience)
  - Following dosing, incision was sutured closed and bandaged
  - Incision sites will be monitored for signs of infection (erythema, edema, accumulation of fluid, scabbing)

**Histopathology:**

- Hemorrhage, fibrin, inflammation
  - Increased fibrosis/healing along incision line
  - Inflammatory response and fibrosis localized
  - Vascularized fibrous connective tissue, macrophages, giant cells, fibroblasts
  - No giant cells observed in low dose group.
  - Macrophages and giant cells were not present.

### In Rats, LIQ865A was Well-Tolerated with No Adverse Findings

In both Rats and Minipigs, tissue response to LIQ865A was a continuum progressing from the initial injury and an acute cellular response through a granulomatous inflammatory response to wound healing.

**In Minipigs, LIQ865A was Well-Tolerated with No Effect on Healing**

In both Rats and Minipigs, tissue response to LIQ865A was a continuum progressing from the initial injury and an acute cellular response through a granulomatous inflammatory response to wound healing.

**CONCLUSION**

LIQ865A, a slow release microparticle formulation of bupivacaine, is well-tolerated and does not interfere with wound healing after subcutaneous dosing in rats and minipigs.

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