1. **Objective:**

   1.1. To determine the oral bioavailability of the Sponsor’s test article(s), after intravenous (IV) or oral (PO) administration in male Beagle dogs (Crossover or Non-Crossover).

2. **Required from Sponsor**

   2.1. Test article in powder form or prepared ready for dosing
   2.2. Molecular mass (exact mass) of each test article and its salt form/purity
   2.3. MSDS or handling and storage information, e.g., light sensitive, store at -20°C, etc
   2.4. If applicable, instructions for dose vehicle preparation

3. **Deliverables**

   3.1. *Express Plus* report containing results outlined below:
   
   - A. In-life observations on each dog
   - B. A table containing test article concentrations in plasma samples at each time point
   - C. Plasma concentration vs. time graphs
   - D. A table containing test article concentrations in dosing solutions (N=3)
   - E. A table containing appropriate pharmacokinetic parameters for each dose route
     - a. AUC, half-life, C<sub>max</sub>, t<sub>max</sub> and Mean residence time (MRT) for each dose route
     - b. The clearance and volume of distribution of test article after intravenous administration as applicable
     - c. Bioavailability after oral dosing
   - F. Appendices:
     - a. Animal data sheets
     - b. Analytical methodology

   **NOTE:** Raw bioanalytical data (e.g. chromatograms and analytical method summary) will not be included in the basic report. These can be obtained for an additional fee.

4. **Substrate**

   4.1. Test article in an appropriate dose vehicle suitable for dosing by IV and PO route individually

5. **Assay System**

   5.1. Non-naïve, male Beagle dogs weighing between 8 to 14 kilograms
   5.2. Animals will be fasted overnight prior to dosing and until 4 hours post-dose
   5.3. Water is offered ad libitum to the dogs

6. **Assay Conditions**
6.1. Analytical method development
A. MS optimization for suitable sensitivity
B. LC evaluation for suitable specificity
C. Standard curve with 6-8 points

6.2. In-life study design
A. Each test article will be assessed through two dose routes, intravenous (IV) and oral (PO)
   a. Non-Crossover: N=3 per dose group per test article (N=6 dogs total)
   b. Crossover: N=3 dogs per leg per test article (N=3 dogs total)
      - Leg 1: Intravenous (IV)
      - Leg 2: Oral (PO)

B. Blood will be sampled from jugular vein (or other suitable vessel) at multiple time points outlined in study design table below
   a. Blood samples will be collected into tubes containing TBD anticoagulant and processed according to the study protocol.
   b. Plasma samples will be prepared and snap frozen. Samples will be stored at -70°C until analysis

C. LC-MS/MS analysis of test article in plasma samples

Table 1: Study design (Non-Crossover)

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Article</th>
<th>Dose Route</th>
<th>N=</th>
<th>Dose (mg/kg)</th>
<th>Dose Conc. (mg/mL)</th>
<th>Dose Rate (mL/kg)</th>
<th>Vehicle</th>
<th>Blood Sampling Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TBD</td>
<td>IV</td>
<td>3</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>Pre-dose, 15, 30 min, 1, 2, 4, 8, and 24 hours post dose</td>
</tr>
<tr>
<td>2</td>
<td>TBD</td>
<td>PO</td>
<td>3</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>Pre-dose, 15, 30 min, 1, 2, 4, 8, and 24 hours post dose</td>
</tr>
</tbody>
</table>

Table 2: Study design (Crossover)

<table>
<thead>
<tr>
<th>Leg</th>
<th>Test Article</th>
<th>Dose Route</th>
<th>N=</th>
<th>Dose (mg/kg)</th>
<th>Dose Conc. (mg/mL)</th>
<th>Dose Rate (mL/kg)</th>
<th>Vehicle</th>
<th>Blood Sampling Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TBD</td>
<td>IV</td>
<td>3</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>Pre-dose, 15, 30 min, 1, 2, 4, 8, and 24 hours post dose</td>
</tr>
</tbody>
</table>

Minimum 7 Day Washout

<table>
<thead>
<tr>
<th>Leg</th>
<th>Test Article</th>
<th>Dose Route</th>
<th>N=</th>
<th>Dose (mg/kg)</th>
<th>Dose Conc. (mg/mL)</th>
<th>Dose Rate (mL/kg)</th>
<th>Vehicle</th>
<th>Blood Sampling Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>TBD</td>
<td>PO</td>
<td>3</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>Pre-dose, 15, 30 min, 1, 2, 4, 8, and 24 hours post dose</td>
</tr>
</tbody>
</table>
6.3. Sample analysis

A. Determine the concentrations of test article in dosing solutions and incurred samples using a generic LC-MS/MS method with a minimum 6-8 point calibration curve

B. The analytical rigor does not include a pre-study validation, and QCs will not be used for sample analysis

C. Dosing solutions will be normalized in matched matrix (dog plasma) and analyzed (n=3) in the same analytical batch as the incurred samples

D. Non-compartmental analysis is used to determine PK parameters for each test article

E. Batch Acceptance Criteria
   a. At least 60% of the calibration standards must be within ±20% of the nominal values to accept the analytical run, except at the Lower Limit of Quantification (LLOQ), where ±25% is acceptable