**Purposes**

- ON 123300 is a novel third generation cyclin-dependent kinases 4/6 (CDK4/6) inhibitor with dual inhibition of c-MYC activated kinases ARK5 controlling cellular metabolism and survival with low nanomolar potency.
- ON 123300 has the potential to be effective in patients developing resistance to second generation CDK4/6 inhibitor compounds.
- CYP450 reaction phenotyping studies suggested that ON 123300 is susceptible to metabolism by CYP3A4 and CYP2C8.
- CYP3A4 constitutes about 30% of the total CYP 450 in liver. They are found to be more actively secreted in males compared to females in certain species.
- This study was undertaken to investigate the gender differences in the metabolism of ON 123300 in rats, a preclinical toxicological species.

**Methods**

- In vitro metabolism experiments were performed in rat liver microsomes from male and female donors.
- ON 123300 (final 10 μM) was incubated with microsomes, and samples (100 μl) were withdrawn at specified incubation times over 60 minutes and immediately quenched and centrifuged.
- The supernatant was analyzed for ON 123300 and its metabolites by HPLC.
- An in vivo pharmacokinetic study was performed in male and female SD rats using intravenous (bolus over 30 sec; n = 3/gender) or oral route of administration (n=5/gender). Intravenous doses were 5 mg/kg and 10 mg/kg; whereas oral dose was 100 mg/kg. Blood samples were collected over 4 hours and 24 hours for IV and oral route of administration, respectively.
- ON 123300 plasma concentrations were measured by LC-MS/MS method and PK parameters were estimated by non-compartmental analysis.

**Results**

Table 1: ON 123300 pharmacokinetic parameters from in vivo liver microsomal metabolism study from male and female rat donors

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2 (min)</td>
<td>10.8</td>
<td>38.2</td>
</tr>
<tr>
<td>Cmax (μg/min/mg)a</td>
<td>130</td>
<td>36.7</td>
</tr>
<tr>
<td>AUC0-24 (μg/min/mg)a</td>
<td>239</td>
<td>67.5</td>
</tr>
<tr>
<td>Predicted Cl in vivo (μL/min/kg)a</td>
<td>2.33</td>
<td>0.670</td>
</tr>
</tbody>
</table>

*a data presented as the average of duplicate experiments

- Estimated using equation $\text{Cmax} = \frac{\text{Qf}}{\text{Cl} \times \text{f} \times \text{Cl}_{\text{in}}, \text{in vivo} \times \text{Qf}}$
- Estimated using equation $\text{AUC0-24} = \frac{\text{Cl} \times \text{MPPGL} \times \text{Wt}}{\text{fu}}$ where MPPGL (microsomal protein per gram of liver) is 46 mg/g and average liver weight is 10 g (normalized by average body weight of 0.25 kg)$^a$. $^a$Estimated using equation $\text{t1/2} = \frac{0.693 \times \text{Vd}}{\text{Cl}}$ where fu is the fraction of drug unbound in blood ($f_u = 0.01$) and Q is the rat hepatic blood flow (85 mL/min/kg).

Table 3: ON 123300 pharmacokinetic parameters following oral administration (100 mg/kg) to male and female rats

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>321 ± 58.9</td>
<td>1253 ± 590</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>2.3 ± 0.8</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>AUC0-24 (ng/hr/mL)</td>
<td>1965 ± 749</td>
<td>5617 ± 1914</td>
</tr>
<tr>
<td>Cl/F (mL/min/Kg)</td>
<td>971 ± 329</td>
<td>372 ± 228</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td>1.9 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
</tbody>
</table>

* data presented as mean ± SD of 5 animals per group

**Conclusions**

- The in vivo intrinsic clearance was ~3.5 fold higher in male liver microsomes compared to female liver microsomes suggesting a differential expression of CYP’s responsible for the metabolism of drug.
- The observed clearance (Table 2) in male and female rats was significantly higher than the predicted in vivo clearance based on intrinsic clearance data from the in vitro studies in liver microsomes (Table 1). This may be due to extrahepatic drug metabolism, active drug uptake into the liver and renal elimination of unchanged drug.
- Drug exposure was dose proportional. The clearance in male rats (~90 mL/min/kg) approximated rat hepatic blood flow (85 mL/min/kg) suggesting that the compound has a high hepatic extraction ratio.
- Consistent with in vivo liver microsome study, ON 123300 displayed significantly higher exposure (~3 fold increase of AUC) in female rats compared to male rats after oral administration.
- Gender differences in the pharmacokinetics of the drug should be taken into account while selecting the relevant species for toxicological evaluation of the compound; and designing the dosing strategy for further development.

**References**