Preclinical characterization of THE-630, a next-generation inhibitor for KIT-mutant gastrointestinal stromal tumors (GIST)

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Gastrointestinal stromal tumors (GIST):
Treatment landscape and unmet needs

- Most common sarcoma of the GI tract
  - ~4,000-6,000 new GIST cases in US each year
- ~80% of cases driven by activating mutations in KIT
- Outcomes are poor in 2L and beyond
- Unmet needs: 5L – and superior option in earlier lines

*Avapritinib is approved in the US for GIST patients with PDGFRA exon18 mutations only

Abbreviations: ORR (objective response rates); mPFS (median progression free survival); m (months); L (line)
Sources: GLEEVEC [package insert]; SUTENT [package insert]; STIVARGA [package insert]; QINLOCK [package insert]
There remains a need for a pan-KIT inhibitor for advanced GIST patients

Genetics of drug-sensitivity are well understood

- 2 major classes of activating mutations
  - Imatinib, but not sunitinib, has reduced activity against Ex9 class
- ~80% of relapse cases driven by secondary resistance mutations in KIT
  - Most occur in ATP binding pocket (Exon 13/14) or Activation loop (Exon 17/18)
  - Most patients have more than 1 resistance mutation
- No drug has potent activity against all classes of activating and resistance mutations

Sources: Corless, C. et al. (2014) Mod. Pathol 27, S1-S16; Preclinical potency assessment also based on studies reported in this poster
Predictive preclinical modeling of clinical drug sensitivity using engineered BaF3 cells

- TKI potency was evaluated using a panel of BaF3 cell lines engineered to express KIT variants shown in the Table (next slide)
- Viability assays (72 h) were performed using culture medium supplemented with physiological concentrations of human serum albumin (341 μM) and α1-acid glycoprotein (1 mg/mL) – to incorporate functional effects that binding to human serum proteins has on target inhibition
- Ripretinib and its active metabolite DP-5439 were tested separately and results averaged
- IC$_{50}$s were related to average TKI concentration (C$_{av}$; AUC/24 h) in patients at the approved dose
  - Imatinib$^1$ C$_{av}$ 3377 nM
  - Sunitinib$^2$ C$_{av}$ 136 nM
  - Ripretinib$^3$ C$_{av}$ 2126 nM (sum of ripretinib and DP-5439 levels)
- Cellular IC$_{50}$ in presence of human serum proteins related to patient C$_{av}$ to predict clinical efficacy
  - KIT variants inhibited with IC$_{50}$s substantially below the patient C$_{av}$ are predicted to be highly sensitive
  - KIT variants inhibited with IC$_{50}$s substantially above the C$_{av}$ are predicted to be highly resistant
  - KIT variants inhibited with IC$_{50}$s close to the C$_{av}$ (± ~2-fold) are predicted to be associated with stable disease, but ultimate tumor progression

Genotype of KIT variants in BaF3 cells

<table>
<thead>
<tr>
<th>KIT Variant Abbreviation</th>
<th>Primary Mutation</th>
<th>Secondary Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Location</td>
<td>Genotype</td>
</tr>
<tr>
<td>Ex11Del</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ex11Del + V654A</td>
<td>Exon 11 (JM)</td>
<td>Del 557_558</td>
</tr>
<tr>
<td>Ex11Del + T670I</td>
<td>Exon 13 (ATP pocket)</td>
<td>V654A</td>
</tr>
<tr>
<td>Ex11Del + D816G</td>
<td>Exon 14 (ATP pocket)</td>
<td>T670I</td>
</tr>
<tr>
<td>Ex11Del + D816H</td>
<td>Exon 17 (A-loop)</td>
<td>D816G</td>
</tr>
<tr>
<td>Ex11Del + D820A</td>
<td></td>
<td>D820A</td>
</tr>
<tr>
<td>Ex11Del + D820G</td>
<td></td>
<td>D820G</td>
</tr>
<tr>
<td>Ex11Del + N822K</td>
<td></td>
<td>N822K</td>
</tr>
<tr>
<td>Ex11Del + Y823D</td>
<td></td>
<td>Y823D</td>
</tr>
<tr>
<td>Ex11Del + A829P</td>
<td>Exon 18 (A-loop)</td>
<td>A829P</td>
</tr>
<tr>
<td>Ex9Ins</td>
<td>Exon 9 (ECD)</td>
<td>Ins (502AY)</td>
</tr>
<tr>
<td>Ex9Ins + V654A</td>
<td>Exon 13 (ATP pocket)</td>
<td>V654A</td>
</tr>
<tr>
<td>Ex9Ins + D816H</td>
<td>Exon 17 (A-loop)</td>
<td>D816H</td>
</tr>
</tbody>
</table>

Viability/signaling assays in cancer cell lines

- TKI potency in cancer cell lines and parental BaF3 cells was evaluated using standard cell culture medium
- Immunoblot analyses were performed on GIST-T1 cells treated for 2 hr

Efficacy studies

- GIST-T1 or engineered BaF3 cells were implanted subcutaneously into the right flank of female CB-17 SCID mice and TKIs administered orally when tumors reached ~140 mm³.
  - Sunitinib 20 mg/kg QD
  - Ripretinib 50 mg/kg BID
  - THE-630 10-25 mg/kg QD
- All dose levels were well-tolerated with no clinical signs observed

Kinase assays (Nanosyn)

- Single point (0.1 μM) screen was performed on 300 kinases (Km of ATP)
- IC_{50}s were determined for kinases inhibited by >50% (1 mM ATP)
Predictive preclinical modeling of clinical drug sensitivity using engineered BaF3 cells

- Mutant classes predicted to be associated with resistance or minimal efficacy – i.e., since exposure in patients (represented by $C_{av}$) does not substantially exceed the mutant $IC_{50}$
  - Imatinib: Ex13/14 and Ex17/18
  - Sunitinib: Ex17/18
  - Ripretinib: Ex13/14 and Ex9Ins primary ± secondary
THE-630 has the profile of a pan-KIT inhibitor for GIST in vitro

Pan-KIT inhibitory profile

• Potent cellular activity against
  - Ex11Del ± 9 resistance mutations (Ex13/14/17/18)
  - Ex9Ins ± 2 resistance mutations (Ex13/17)
  - PDGFRA V561D (IC$_{50}$ < 5 nM)

• 100 nM target C$_{av}$ in patients for pan-KIT mutant activity

Selectivity profile

• 46/300 kinases inhibited by >50% at 0.1 μM
  - Ripretinib: 44/300

• 28 kinases inhibited with IC$_{50}$ within 100-fold of KIT IC$_{50}$ (0.5 nM)
  - Ripretinib: 27 (KIT IC$_{50}$ 1.5 nM)

• THE-630 not designed to potently inhibit PDGFR D842V (GIST) and KIT D816V (mastocytosis) activating mutations
  - IC$_{50}$ > 100 nM in BaF3 cellular assays
THE-630 is highly active in cancer cell lines with activating mutations in KIT

Potent activity against KIT Ex11Del, N822K (Ex17) and D816Y (Ex17) activating mutations

<table>
<thead>
<tr>
<th>Cell line</th>
<th>KIT mutation</th>
<th>Exon</th>
<th>Tissue</th>
<th>Imatinib</th>
<th>Sunitinib</th>
<th>Ripretinib</th>
<th>THE-630</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIST-T1</td>
<td>Ex11Del</td>
<td>11</td>
<td>Human GIST</td>
<td>30</td>
<td>13</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Kasumi</td>
<td>N822K</td>
<td>17</td>
<td>Human AML</td>
<td>832</td>
<td>51</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>P815</td>
<td>D816Y</td>
<td>17</td>
<td>Murine mastocytoma</td>
<td>2599</td>
<td>195</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>BaF3**</td>
<td>N/A</td>
<td></td>
<td>Murine pro B cells</td>
<td>8434</td>
<td>2311</td>
<td>9545</td>
<td>2379</td>
</tr>
</tbody>
</table>

*Viability assays performed in the absence of human serum proteins

**Untransformed parental BaF3 cells grown in the presence of IL3

THE-630 cell killing associated with inhibition of KIT and downstream signaling

GIST-T1 cells treated for 2 hr
THE-630 is highly efficacious in tumor models containing KIT Ex11 and Ex9 activating mutations

**Ex11Del (primary mutation)**

- Vehicle QD
- Vehicle BID
- Ripretinib 50 mg/kg BID
- Sunitinib 20 mg/kg QD
- THE-630 10 mg/kg QD

**Ex9Ins (primary mutation)**

- Vehicle QD
- Vehicle BID
- Ripretinib 50 mg/kg BID
- Sunitinib 20 mg/kg QD
- THE-630 20 mg/kg QD

GIST-T1 model

BaF3 model
THE-630 is highly efficacious in tumor models containing KIT Ex13, 17, and 18 resistance mutations

Ex11Del + V654A (Ex13)
- Vehicle QD
- Vehicle BID
- Ripretinib 50 mg/kg BID
- THE-630 25 mg/kg QD

Ex11Del + N822K (Ex17)
- Vehicle QD
- Sunitinib 20 mg/kg QD
- THE-630 20 mg/kg QD

Ex11Del + D820A (Ex17)
- Vehicle QD
- Vehicle BID
- Ripretinib 50 mg/kg BID
- THE-630 20 mg/kg QD

Ex11Del + A829P (Ex18)
- Vehicle QD
- Vehicle BID
- Ripretinib 50 mg/kg BID
- Sunitinib 20 mg/kg QD
- THE-630 20 mg/kg QD

All studies: BaF3 model
THE-630 is highly efficacious in tumor models containing all classes of KIT activating and resistance mutants

- **THE-630 highly active against**
  - Most common primary activating mutants (Ex11Del and Ex9Ins)
  - Most common ATP-binding pocket mutant (V654A)
  - All 3 activation loop mutants tested (N822K, D820A, A829P)

- **THE-630 efficacy ≥ ripretinib against all mutants tested**

<table>
<thead>
<tr>
<th></th>
<th>Ex9Ins</th>
<th>Ex11Del</th>
<th>V654A (Ex13)</th>
<th>N822K (Ex17)</th>
<th>D820A (Ex17)</th>
<th>A829P (Ex18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunitinib</td>
<td>66% TR</td>
<td>59% TR</td>
<td>91% TR</td>
<td>25% TGI</td>
<td>ND</td>
<td>0% TGI</td>
</tr>
<tr>
<td>Ripretinib</td>
<td>17% TGI</td>
<td>41% TR</td>
<td>25% TGI</td>
<td>ND</td>
<td>1% TR</td>
<td>81% TGI</td>
</tr>
<tr>
<td>THE-630</td>
<td>58% TR</td>
<td>85% TR</td>
<td>86% TGI</td>
<td>83% TR</td>
<td>59% TR</td>
<td>90% TGI</td>
</tr>
</tbody>
</table>

TGI: tumor growth inhibition; TR: tumor regression; ND: Not determined

Preclinical Efficacy: • Stasis/regression • No/minimal TGI
THE-630 has favorable ADMET properties

<table>
<thead>
<tr>
<th>ADME/Safety</th>
<th>THE-630</th>
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<tbody>
<tr>
<td>Plasma protein binding, 1 µM (Rat, Monkey, Human)</td>
<td>95, 90, 95%</td>
</tr>
<tr>
<td>Metabolic clearance (Rat, Monkey, Human LMs)</td>
<td>High, low/mod, low</td>
</tr>
<tr>
<td>CYP IC₅₀s (1A2, 2C9, 2C19, 2D6, 3A4)</td>
<td>&gt;20 µM (no TDI)</td>
</tr>
<tr>
<td>hERG (IC₅₀)</td>
<td>&gt;10 µM</td>
</tr>
</tbody>
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Toxicokinetics in NHPs after 14 days of oral daily dosing

- 14 daily doses were tolerated in NHPs at exposures that matched (2 mg/kg) or exceeded, by 2.5-fold (4 mg/kg), the target $C_{av}$ for pan-KIT activity

AACR 2021 Poster 1292
Conclusions

• THE-630 has the profile of next-generation, pan-KIT inhibitor for GIST
  - Potent activity in cellular assays and strong efficacy in tumor models against both classes of activating mutations (Ex9 and Ex11) and both classes of resistance mutations (Ex13/14 and Ex17/18)
  - Good selectivity profile

• IND-enabling studies are underway based on promising nonclinical data
  - Favorable ADME profile
  - Target pan-KIT exposure levels achieved at tolerable doses in 14-day rat and NHP pilot studies

• A phase 1 clinical trial of THE-630 in patients with GIST is planned to begin in the second half of 2021