Fyn & Pyk2 Kinase Inhibition for Alzheimer's Disease

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Synapse Damage Pathway in Alzheimer’s Disease

• No Disease-Modifying Therapy for AD
• Pharma focus and failures relate to Aβ levels *per se*
• Amyloid β Oligomers (Aβo) damage synapses in AD to cause symptoms
• Discovered molecular cascade
• Pyk2 genetically linked to AD risk
• Fyn or Pyk2 kinase blockade restores synapses and memory to AD mice

*Biology documented by our lab in multiple high profile publications and supported by numerous competitive NIH grants*
Efficacy of Fyn and Pyk2 Inhibition in Preclinical Models

- We have just completed a Phase 2a Trial with Fyn Inhibitor for AD with AZ and NIH
- Limited dose due to platelet suppression and interstitial pulmonary fibrosis, and Fyn null phenotypes
- Therapeutic index of 2 extrapolated from mouse
- Data analyses now in progress, to be reported in July

Similar rescue of memory and synapses in Pyk2 null state (data not shown)
Fyn/Pyk2 Inhibition with Robust Therapeutic Index

- Dose-limiting side-effects with complete single kinase inhibition
- Synergistic cross-phosphorylation and co-activation of Fyn and Pyk2
- Partial inhibition of both enzymes anticipated to be efficacious with broad therapeutic window

- Option A: one compound with balanced dual inhibition
- Option B: Proprietary Pyk2 inhibitor compound +/- existing Fyn inhibitors

- Blavatnik Project to Develop SAR and IP for Dual Pyk2/Fyn or Pyk2 inhibitors
# Current Blavatnik Fund & CRL Contract

<table>
<thead>
<tr>
<th>Description</th>
<th>Complete</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>1: Virtual screen using commercial deck against Fyn and Pyk2</td>
<td>Dec-17</td>
<td>CRL</td>
</tr>
<tr>
<td>2: Compound provision for 500 selected compounds</td>
<td>Feb-18</td>
<td>CRL</td>
</tr>
<tr>
<td>3: Biochemical assay development for Fyn and Pyk2</td>
<td>Feb-18</td>
<td>CRL</td>
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<tr>
<td>4: Compound handling</td>
<td>Feb-18</td>
<td>CRL</td>
</tr>
<tr>
<td>5: Profile 100 compounds at 1 and 10 µM in both assays</td>
<td>Mar-18</td>
<td>CRL</td>
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<tr>
<td>6: Hit expansion by purchase &amp; synthesis of close analogues, including biochemical retest</td>
<td>Ongoing</td>
<td>CRL</td>
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<tr>
<td>7: Selectivity panel, 20-30 major kinases at 1 µM</td>
<td></td>
<td>CRL</td>
</tr>
<tr>
<td>8: Cellular assay development for Fyn and Pyk2</td>
<td>Initiated</td>
<td>CRL</td>
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<tr>
<td>9: 1 week compound profiling cellular assays</td>
<td></td>
<td>CRL</td>
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<tr>
<td>10: DMPK analysis for 10 compounds</td>
<td></td>
<td>CRL</td>
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<tr>
<td>11: Test dual Fyn/Pyk2 kinase inhibitors with highly predictive synapse-specific preclinical <em>in vitro</em> AD models</td>
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<td>Yale</td>
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</tbody>
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*Test synapse stability by imaging and plasticity by LTP electrophysiology*
## Virtual Screening

<table>
<thead>
<tr>
<th>Method</th>
<th>Hits selected</th>
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</thead>
<tbody>
<tr>
<td>Structure based VS (2DQ7)</td>
<td>889</td>
</tr>
<tr>
<td>Structure based VS (3FZP)</td>
<td>219</td>
</tr>
<tr>
<td>Structure based VS (3FZS)</td>
<td>47</td>
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<tr>
<td>E-pharmacophore</td>
<td>77</td>
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<tr>
<td>Ligand based pharmacophore screening</td>
<td>495</td>
</tr>
<tr>
<td>Hit expansion</td>
<td>79</td>
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<tr>
<td>Bayesian classification</td>
<td>90</td>
</tr>
<tr>
<td>ROCS 3-D shape screening</td>
<td>453</td>
</tr>
</tbody>
</table>

**REMOVED**
- Duplicates
- Hits with
  1. Structural alerts
  2. LogP ≥ 4.5
  3. MW > 450 & MW < 250
  4. Polar Surface Area > 120

**522 HITS**

16 FYN-PYK2 shared

**348 HITS OBTAINED** from VENDORS
Hit Selection from Virtual Screen

FYN-PYK2 shared hit example from E-pharmacophore results

Figure legend: protein amino acids shown in green; ligand based pharmacophore features shown in green (lipophilic features), orange (aromatic moiety), blue (hydrogen bond donor group), red (hydrogen bond acceptor group); excluded volumes indicated with blue spheres and hydrogen bonds reported with yellow, dashed lines.
**Assay Development: ADP Glo™ FYN & Pyk2 assays**

**Assay Performance (Z')**

- **Z' FYN**
- **Z' PYK2**

- **FYN**: mean +/- SD = 0.65 +/- 0.05
- **PYK2**: mean +/- SD = 0.66 +/- 0.06

**Expected potencies for known Fyn and Pyk2 inhibitors**

Cell-based assays being developed in Jurkat cells with endogenous Fyn and Pyk2
Fyn/Pyk2 Inhibitors

Inhibition at 10µM

% Fyn inhibition @ 10µM

% Pyk2 inhibition @ 10µM
Hit expansion

Selected compounds for hit expansion

Series 1: Aza-indole

- **502413** could be developed for PYK2 inhibition and possibly dual inhibition
- Synthetic tractability:
  - Cores structures (Azaindole-R1) can be easily accessed
  - Large arrays can be generated from reductive amination to introduce R2

Series 2: Amino-pyrazole

- **502468** could be developed as dual inhibitor
- Synthetic tractability: arrays of analogues can be generated from amide coupling
A rapid hit expansion has been completed
The central ring SAR was probed using unsaturated piperidine and piperazine
Series 2: CR000502468 Hit expansion

Amino-pyrazole scaffold

- Chemistry and purification method have been validated to synthesise the desired targets

CR000502468
FYN IC₅₀ 16 µM
PYK2 IC₅₀ 23 µM
A focused set of compounds based on SAR knowledge of the pyrazole urea scaffold will also be synthesised.
• **Series 1:**
  - 45 compounds were synthesised
  - Unsaturated piperazine is critical to retain PYK2 potency
  - Small structural changes on the phenyl substituents can alter potency against PYK2 and FYN

• **Series 2:**
  - 10 compounds have been synthesised and characterised
  - This array will be tested shortly against FYN and PYK2
  - 502468 will be modified based on structure activity knowledge of pyrazole ureas

**Hit Expansion Summary**

CR000502413

CR000502468

PYK2 IC$_{50}$ 6 nM
Anticipated Results by September

- Evaluate focused compound library and analogues for potency and selectivity to develop SAR profile
- Kinase selectivity results
- Compound activity in cellular assay
- Pharmacologic ADME properties of inhibitors
- IP protection
Proposed Blavatnik Continuation

• Decision point before September
  – Option A (Dual) *versus* Option B (Proprietary Pyk2)
  – Advance to DMPK work *versus* further SAR

• Use of Second Year ($300K) Funds
  – PK/PD, dose-ranging toxicology for lead(s)
  – Further medicinal chemistry, as indicated
  – Continued contract with CRL

  – Test synapse and AD mouse memory at Yale
    (no additional cost)
Synapse Damage Pathway in Alzheimer’s Disease

- First Disease-Modifying Therapy
- Protect synapses in AD
- Pyk2 genetically linked to AD risk
- Fyn or Pyk2 kinase blockade restores synapses and memory to AD mice

- Develop SAR and IP for Dual Pyk2/Fyn or Pyk2 inhibitors
- Progress during last 6 months
- Continue advance of chemistry and IP to partnership

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