Knopp Biosciences

The re-emergence of Kv7 drug discovery

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**NEW TARGET:**

Bristol-Myers Squibb Ion Channel Group (USPTO, 1996)

*The present invention relates to KCNQ proteins defining potassium channels.*


*This finding in BFNC provides direct evidence that defects in potassium channels are involved in the mammalian epilepsy phenotype*

**NOVEL AED:**


*D-23129 thus presents an orally active, safe, broad spectrum anticonvulsant agent, structurally unrelated to anticonvulsants currently used.*

*K+ channel opening effect contributes to the anticonvulsant activity of retigabine.*

*Confirmed that retigabine acts on KCNQ channels. A unique AED target, linked to genetic forms of epilepsy.*
Pharmacology: Ezogabine preclinical has a broad anti-seizure efficacy profile in multiple models

<table>
<thead>
<tr>
<th>Model</th>
<th>Translational Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal electroshock: mouse, rat</td>
<td>Generalized tonic-clonic seizure</td>
</tr>
<tr>
<td>s.c. Pentylenetetrazole-induced seizure, mouse</td>
<td>Generalized tonic-clonic seizure</td>
</tr>
<tr>
<td>NMDA-induced seizure, mouse</td>
<td>Generalized tonic-clonic seizure</td>
</tr>
<tr>
<td>Amygdala kindling, rat</td>
<td>Partial epilepsy</td>
</tr>
<tr>
<td>Rapid hippocampal kindling, immature rat</td>
<td>Pediatric epilepsy</td>
</tr>
<tr>
<td>Lamotrigine-resistant amygdala kindled rat</td>
<td>Pharmacoresistant epilepsy (carbamazepine, phenytoin, topiramate-resistant)</td>
</tr>
<tr>
<td>Kainic acid-induced status epilepticus</td>
<td>Chronic pathology of temporal lobe epilepsy, status epilepticus</td>
</tr>
<tr>
<td>6 Hz psychomotor seizure</td>
<td>Pharmacoresistant epilepsy</td>
</tr>
<tr>
<td>Genetic Absence Epilepsy Rats of Strasbourg (GAERS)</td>
<td>Generalized, absence seizure</td>
</tr>
<tr>
<td>Genetically epilepsy-prone rats (GEPRs)</td>
<td>Auditory-induced seizure</td>
</tr>
<tr>
<td>DBA/2 mouse</td>
<td>Auditory-induced seizure (Kir4.1 mutant)</td>
</tr>
<tr>
<td>Human brain tissue resected from patients with intractable epilepsy</td>
<td></td>
</tr>
<tr>
<td>1. Spontaneously rhythmic sharp waves</td>
<td></td>
</tr>
<tr>
<td>2. Induced seizure activity</td>
<td></td>
</tr>
<tr>
<td>Ex-vivo - pharmacoresistant epilepsy</td>
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</table>

Ezogabine
Commercially available Kv7 modulators for evaluating a diverse set of smooth muscle and central nervous system disorders

<table>
<thead>
<tr>
<th>Kv7.2/7.3 Openers/Activators</th>
<th>Blockers/Inhibitors</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ezogabine</td>
<td>1. XE-991</td>
<td>1. <strong>Epilepsies</strong></td>
</tr>
<tr>
<td>2. Flupirtine</td>
<td>2. Linopirdine</td>
<td>2. Pain</td>
</tr>
<tr>
<td>3. ICA-27243</td>
<td>3. 4-Aminopyridine</td>
<td>3. Bladder contractility</td>
</tr>
<tr>
<td>4. ICA-069673</td>
<td></td>
<td>4. Tinnitus</td>
</tr>
<tr>
<td>5. ICA-110381</td>
<td></td>
<td>5. DFNA hearing loss</td>
</tr>
<tr>
<td>6. ML-213</td>
<td></td>
<td>6. Preterm labor</td>
</tr>
<tr>
<td>7. Meclofenamic/Diclofenac</td>
<td></td>
<td>7. Irritable bowel</td>
</tr>
<tr>
<td>8. Acrylamide S-1/S-2</td>
<td></td>
<td>8. Hypertension</td>
</tr>
<tr>
<td>9. BMS-204352</td>
<td></td>
<td>9. Paroxysmal Dyskinesia</td>
</tr>
<tr>
<td>10. NS-15370</td>
<td></td>
<td>10. Hypoxic pulmonary vasoconstriction</td>
</tr>
</tbody>
</table>


Goals of the Knopp Kv7.2/7.3 activator program

- Discover and develop best-in-class Kv7.2/7.3 activators
- Capitalize on opportunities for improvement over ezogabine
  - Eliminate chemical instability we hypothesize led to black box warning
    - Avoid anilines in scaffold
  - Improve selectivity: eliminate GABA$_A$ activity
    - Potentially reduce somnolence, fatigue, dizziness
    - Improve therapeutic index
  - Improve dosing regimen
    - Ezogabine dosed TID up to 1,200 mg/day due to plasma instability and rapid clearance mechanisms
Pharmacophore-based design approach

- Pharmacophore-based design principles were employed to create novel activator classes
- Scientific and patent literature reviewed to identify crucial pharmacophores
- Small, focused libraries of several templates were designed to discover new classes of Kv7 activators
  - *Significantly differentiated in structure from ezogabine-based compounds*

General templates from journal and patent literature

Molecular parameters (e.g. MW, cLogP, tPSA, H-bond donors) considered in the designs of Knopp’s novel Kv7 activators

In vitro predictive ADME screening employed to optimize physicochemical properties and correlate with in vivo PK and efficacy
Simplified screening funnel

In house ion channel screens

Primary Screen
Thallium Flux EC$_{50}$
Kv7.2/7.3 and Kv7.4

Electrophysiological validation (QPatch)
Kv7.2/7.3 and Kv7.4

Cardiac profiling (QPatch)
hERG and Kv7.1/KCNE1 screen

Profiling on Kv7.3/7.5
Thallium Flux and QPatch

Tier I/II ADME

PK Experiments

Efficacy and Tolerability

Tier III ADME

Advanced Assays
Primary Screen: thallium flux assay

- Thallium (TI⁺) is used as a surrogate marker for K⁺ current and activity is measured by TI⁺ interacting with an intracellular dye

- Compounds are screened against Kv7 expressing cell lines (8 pt CRC, 5 nM to 10 µM)

- Rapid EC₅₀ and Eₘₐₓ data collection

![Thallium flux assay diagram]

https://tools.thermofisher.com/content/sfs/manuals/MAN0016084_FluxOR_II_Green_UG.pdf
QPatch: development and validation in house outline

- Kv7 activity confirmed activity with a functional assay
- Additional insights gained on mechanism of channel kinetics
- Ability to have a high reproducibility of response

Activity measurement, represented as fold increase $K^+$ current over saline control ($I/I_0$)

Kinetic measurements obtained (Tau). Tau behaves independent of activation and may influence efficacy

Voltage Protocol

- Holding -80 mV
- Activating -30 mV
- Deactivating -120 mV
A range of activities against Kv7.2/7.3 and Kv7.4

**EC$_{50}$ determined in a fluorescence-based assay**

- **Kv7.2/7.3**
  - Wide variations in potency observed
  - Variations in efficacy are less common

- **Kv7.4**
  - Both potency and efficacy exhibit substantial variation
  - Assay in screening format not set up to detect inhibition
QPatch activity categorization process

<table>
<thead>
<tr>
<th>Group</th>
<th>Kv7.2/7.3 Activity</th>
<th>Kv7.4 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>2</td>
<td>EZG-like</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>4</td>
<td>Low</td>
<td>EZG-like</td>
</tr>
<tr>
<td>5</td>
<td>EZG-like</td>
<td>EZG-like</td>
</tr>
<tr>
<td>6</td>
<td>High</td>
<td>EZG-like</td>
</tr>
<tr>
<td>7</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>8</td>
<td>EZG-like</td>
<td>Low</td>
</tr>
<tr>
<td>9</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>10</td>
<td>Low</td>
<td>Inhib</td>
</tr>
<tr>
<td>11</td>
<td>EZG-like</td>
<td>Inhib</td>
</tr>
<tr>
<td>12</td>
<td>High</td>
<td>Inhib</td>
</tr>
</tbody>
</table>
Group categorization by 7.2/7.3 activity relative to 7.4

- Groups 3, 5, and 7
  - “Scaled” version of ezogabine; some activity on both ion channels
- Groups 6, 8, and 9
  - Kv7.2/7.3 dominant profiles
  - Potentially useful for epilepsy with reduced side effects compared to ezogabine
  - Knopp is actively investigating and developing compounds from this group
- Groups 1, 2, and 4
  - Kv7.4-dominant profiles
- Groups 10, 11, and 12
  - Kv7.4 inhibitors with varied Kv7.2/7.3 activities
Group 8: Knopp IND candidate KB-3061 for KCNQ2-EE is selective against GABA$_A$ and stable to photooxidation

**GABA$_A$ (α1β3γ2) PAM Assay**

**Stability to photooxidation (VIS and UV)**

Blue: EC$_{10}$ GABA  
Black: 10µM KB-003061-01 + EC$_{10}$ GABA  
Red: 10µM Diazepam + EC$_{10}$ GABA
KB-3061 is more potent than ezogabine on Kv7.2/7.3 expressing cells

**Voltage-gated sweeps**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Current [pA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
<tr>
<td>3 µM 3061</td>
<td>4000</td>
</tr>
<tr>
<td>3 µM EZO</td>
<td>2000</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
</tbody>
</table>

**Voltage Protocol**

- Holding -80 mV
- Activating -30 mV
- Deactivating -120 mV

**Ligand-gated single-cell perfusion mode (1 µM)**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Current [pA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
<tr>
<td>Ezogabine</td>
<td>4000</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
<tr>
<td>KB-3061</td>
<td>4000</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
</tr>
</tbody>
</table>

Displayed Agent periods:
- E2020 (3.4 µM ezogabine)
- KB-3061 (3.0 µM)
- Saline

Displayed perfusion periods:
- E2020 (3.4 µM ezogabine)
- KB-3061 (3.0 µM)
- Saline
KB-3061 is potent in a rat MES model with a wide TI

**KB-3061 in rat MES**

- **ED50**: ~1.1 mg/kg
- **TD50**: > 30 mg/kg

**Ezogabine in rat MES**

- **ED50**: 23 mg/kg
- **TD50**: ~ 50 mg/kg
Group 1 example: Low Kv7.2/7.3 activity, high Kv7.4 activity

<table>
<thead>
<tr>
<th></th>
<th>Kv7.2/7.3</th>
<th>Kv7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPatch $I_3/I_0$</td>
<td>2.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Thallium Flux EC$_{50}$ (µM)</td>
<td>2.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Thallium Flux EC$_{50}$

Kv7.2/7.3 QPatch Trace

Kv7.4 QPatch Trace

- Black: Saline control
- Blue: Compound low 2/3, high 4
Group 12 example: High Kv7.2/7.3 activity, inhibitory Kv7.4 activity

<table>
<thead>
<tr>
<th></th>
<th>Kv7.2/7.3</th>
<th>Kv7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPatch $I_3/I_0$</td>
<td>9.6</td>
<td>0.54</td>
</tr>
<tr>
<td>Thallium Flux $EC_{50}$ (µM)</td>
<td>0.19</td>
<td>NC</td>
</tr>
</tbody>
</table>

**Thallium Flux $EC_{50}$**

**Kv7.2/7.3 QPatch Trace**

**Kv7.4 QPatch Trace**

- **Saline control**
- **Compound low 2/3, inh 4**
While searching for selective Kv7.2/7.3 activators, Knopp has identified a wide range of combinations of Kv7.2/7.3 and Kv7.4 activity.

Among compounds screened against both Kv7.2/7.3 and Kv7.4:
- 560 had an ezogabine-like subtype activation profile (Groups 3, 5, and 7)
- 333 had a Kv7.4-dominant subtype activation profile (Groups 1, 2, and 4)
- 181 had a Kv7.2/7.3-dominant subtype activation profile (Groups 6, 8, and 9)
- 90 had a Kv7.4-inhibitory subtype activation profile (Groups 10, 11, and 12)

Kv7 library could be useful as a tool-set for investigating Kv7 pharmacology
- An opportunity to correlate sub-channel drug profiles with cell and organ specific targets
Acknowledgements

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