“Executing on Na\textsubscript{V} Drug Discovery with the IonWorks Barracuda® Automated Electrophysiology Platform”

David Rock, Ph.D.
Essen Discovery Services
Business Model

“Flexible Access to our Technology and Experience to Drive Drug Discovery”

Ion Channel Services
Live Cell Services
Drug Discovery

Voltage- & Ligand-gated Ion Channel Screens

Cell Player® Assays
Angiogenesis
Cell Migration
Apoptosis
NeuroTrack

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Na\textsubscript{\textit{V}} Drug Discovery Background and Challenges

- Na\textsubscript{\textit{V}} channels mediate regenerative action potentials – responsible for conducting electrical information within excitable cells
- Multiple subtypes of Na\textsubscript{\textit{V}} channels with differential expression, biophysical properties and sensitivity to TTX
- Small molecule Na\textsubscript{\textit{V}} blockers are used as local anesthetics, analgesics, antiarrhythmics, anticonvulsants, mood stabilizers
- Human genetic evidence and preclinical data supports involvement of Na\textsubscript{\textit{V}} channels in pain signaling, particularly Na\textsubscript{\textit{V}}1.7 and Na\textsubscript{\textit{V}}1.8
- Therapeutic efficacy/safety window achieved mainly by state-dependent inhibition; key selectivity target - Na\textsubscript{\textit{V}}1.5 block associated with CV side-effects
- Challenge - increase efficacy with reduced side effects (CV and CNS)
IonWorks®: High Throughput E-Phys Platforms for Ion Channel Electrophysiology

**Impact – IC Discovery Project support with Plate-based V-clamp Ephys Pharmacology**

- Voltage- and Ligand-gated IC targets
- Focused Library Screening – ‘Targeted HTS’
- Hit/Lead Evaluation and SAR Support

Mechanistic Screening – State-Dependent Inhibition
Subtype Selectivity/Off Target Pharmacology

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**Throughput**

<table>
<thead>
<tr>
<th>Throughput</th>
<th>high</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

1000’s single pt /day
100’s IC₅₀ s / day

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**Project positioning**

<table>
<thead>
<tr>
<th>HTS</th>
<th>Hit to Lead</th>
<th>SAR</th>
<th>Efficacy/Safety</th>
<th>IND</th>
</tr>
</thead>
</table>

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Ion Channel Reagents/Validated Assays

Voltage-gated Na\(^+\) channels

hNa\(_V\) 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, rat ND7-23

Voltage-gated K\(^+\) channels

hERG, K\(_V\) 1.3, hK\(_V\) 1.5, hKCNQ2/3, hKCNQ1 + minK

Voltage-gated Ca\(^{2+}\) channels

hCa\(_V\) 3.2, hCa\(_V\) 1.2 (optical)

Ligand-gated & other channels

I\(_{CRAC}\) (optical), TREK-1, P2X7, TRPV1
Voltage-gated Na\(^+\) channels: human Na\(_V\) panel

Stable cell lines in HEK background

Raw data traces – IonWorks\textsuperscript{®} single hole recordings
Voltage-gated Na\(^+\) channels: human Na\(_V\) panel
TTX Sensitivity – IonWorks\textsuperscript{®} PPC recordings

<table>
<thead>
<tr>
<th>TTX-Sensitive</th>
<th>TTX-Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Na}_V1.1)</td>
<td>(\text{Na}_V1.5)</td>
</tr>
<tr>
<td>(\text{Na}_V1.3 + \beta1)</td>
<td>(\text{Na}_V1.8 + \beta1)</td>
</tr>
<tr>
<td>(\text{Na}_V1.4 + \beta1)</td>
<td>(\text{Na}_V1.6)</td>
</tr>
<tr>
<td>(\text{Na}_V1.7)</td>
<td>(\text{Na}_V1.7)</td>
</tr>
</tbody>
</table>
Measuring Subtype Selectivity and State-dependent Block of Na\textsubscript{v} Channels with IonWorks®

**Na\textsubscript{v} channel states**

- **Open**
- **Inactivated**
- **Closed**

**Depolarization**

**Repolarization**

Anticonvulsants
Analgesics
Local anaesthetics
Antiarrhythmics

**“State-Independent”**

Subtype-selective

Na\textsubscript{v}1.7
Tetrodotoxin

Tonic (1\textsuperscript{st} pulse)
Inactivated (25\textsuperscript{th} pulse)

<table>
<thead>
<tr>
<th>Conc. (M)</th>
<th>1\textsuperscript{st} Pulse</th>
<th>25\textsuperscript{th} Pulse</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>-8</td>
<td>Tetracaine</td>
<td>Control</td>
<td>32% Inh</td>
</tr>
<tr>
<td>-6</td>
<td>Tetracaine</td>
<td>Control</td>
<td>46% Inh</td>
</tr>
<tr>
<td>-4</td>
<td>Tetrodotoxin</td>
<td>Control</td>
<td>32% Inh</td>
</tr>
</tbody>
</table>

**“State-Dependent”**

Non-selective

Na\textsubscript{v}1.7
Tetracaine

Tonic (1\textsuperscript{st} pulse)
Inactivated (25\textsuperscript{th} pulse)

<table>
<thead>
<tr>
<th>Conc. (M)</th>
<th>1\textsuperscript{st} Pulse</th>
<th>25\textsuperscript{th} Pulse</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>-9</td>
<td>Tetrodotoxin</td>
<td>Control</td>
<td>23% Inh</td>
</tr>
<tr>
<td>-7</td>
<td>Tetrodotoxin</td>
<td>Control</td>
<td>86% Inh</td>
</tr>
<tr>
<td>-5</td>
<td>Tetrodotoxin</td>
<td>Control</td>
<td>86% Inh</td>
</tr>
</tbody>
</table>

**IC\textsubscript{50} (µM)**

<table>
<thead>
<tr>
<th>State</th>
<th>Tonic</th>
<th>Inactivated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic</td>
<td>0.066</td>
<td>0.038</td>
</tr>
<tr>
<td>Inactivated</td>
<td>0.038</td>
<td>0.066</td>
</tr>
</tbody>
</table>

V-clamp protocol

-80 mV holding, 10 Hz 20 msec step to 0 mV

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Measuring Subtype Selectivity and State-dependent Block of Na\textsubscript{v} Channels with IonWorks®

**Na\textsubscript{v} channel states**

- **Open**
- **Closed**
- **Inactivated**

**State-Independent**
- Subtype-selective
- Tetrodotoxin

**State-Dependent**
- Non-selective
- Tetracaine

**V-clamp protocol**

- 80 mV holding, 10 Hz 20 msec step to 0 mV

**Challenge** – combining subtype-selectivity and state-dependence
Naᵥ Panel Screening
2 Pulse Full Inactivation Protocol - Tetracaine

- State-dependent block of Naᵥ1.7 – 10X higher potency for inactivated states
- Similar potency across other subtypes at inactivated channels
Na\textsubscript{V} Panel Screening

2 Pulse Full Inactivation Protocol – “Subtype-selective” compound #1

* Issues with solubility

- Potent Na\textsubscript{V}1.7 inactivated state blocker
- Minimal activity at other subtypes – Na\textsubscript{V}1.6?
Na\textsubscript{v} Panel Screening
2 Pulse Full Inactivation Protocol – “Subtype-selective” compound #2

- Potent Na\textsubscript{v}1.7 inactivated state blocker, resting state block @ high conc
- Potent Na\textsubscript{v}1.6 inactivated state blocker
- Minimal activity at other subtypes
NaV Panel Screening

2 Pulse Full Inactivation Protocol – “Subtype-selective” compound #3

- Very potent NaV1.7 inactivated state blocker, resting state block with IC\textsubscript{50} = 1 µM
- Potent NaV1.6 and NaV1.3 inactivated state blocker, active at NaV1.1 (under evaluation)
- Block at NaV1.1 similar to resting state block @ NaV1.7
- Minimal activity at NaV1.5 and NaV1.8 (TTX-R channels)
Na\textsubscript{v} Panel Screening

2 Pulse Full Inactivation Protocol – Evaluating Subtype-selectivity – four profiles

Inactivated pulse - 10 min incubation
Norm to positive & neg controls, N=4-6
Pluronic/DMSO Vehicle

Tetracaine

Inactivated pulse - 10 min incubation
Norm to positive & neg controls, N=2-4
Pluronic/DMSO Vehicle

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Summary/Future Directions

- Plate-based pharmacology with IonWorks can provide a detailed characterization evaluation of Na$_V$ channel blockers – mechanistic screening and subtype selectivity.
- Next generation small molecule Na$_V$ blockers combine potent state-dependent inhibition of Nav1.7 with varying degrees of subtype selectivity.
- Subtype selectivity can vary dramatically – Na$_V$ panel screening will help to guide SAR.
- Future work focused on extended repeat gating protocols and serum-shift assays to improve translation.
Essen’s Ion Channel Team

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[AMRI Global SmartSourcing]