CRO Services
Why partner with us?

OUR SPECIALTY IS IN VITRO AND IN VIVO SAFETY PHARMACOLOGY

• Contracted by the FDA to provide ion channel screening services in support of the CiPA initiative
• Recognized experts and thought leaders
• Client based study design and context oriented results

WE DON’T JUST PROVIDE DATA, WE PROVIDE ANSWERS

WITH DECADES OF ION CHANNEL AND IN VIVO EXPERIENCE, CYTOBIOSCIENCE IS UNIQUELY POSITIONED TO BE YOUR ONE STOP SHOP FOR SAFETY PHARMACOLOGY

From the CiPA ion channel panel to stem cells to in vivo studies, we carry out all aspects of the safety characterization of your compounds. We also offer GLP capabilities and dose formulation analysis.

We offer higher throughput ion channel screening services using our CytoPatch – the „hands-free“ manual patch clamp platform.

With our portfolio of capabilities, we can support screening of compounds from discovery through IND enabling studies.

We are proud to have been contracted by the FDA to provide ion channel screening services in support of the CiPA initiative.

References for ion channel screening service using CytoPatch:
F1000 Research 3:245 - October 2014
Late cardiac sodium current can be assessed using automated patch-clamp | Chevalier M, Amuzescu B, Gawali V, Todt H, Knott T, Scheel O, Abriel H
Drug Discovery World Spring 2013: 60-66
An automated approach to solving Pharma’s cardiac toxicity conundrum | Van de Waart B, Westerink W, Pineiro Costas N
ELECTRICAL ACTIVITY OF THE HEART

The electrical activity of the heart is manifested at the whole organ level as the waveform known as the electrocardiogram (ECG). This is the result of electrical impulses generated in the sino-atrial node (SAN) conducting throughout the atria, through the atrio-ventricular node (AVN) and then into the ventricle via the His-Purkinje system. Underlying the overall electrical activity of the heart are action potentials generated from each of the electrically excitable cells in the heart. Further dissection of the electrical activity of the heart reveals that a variety of ion channels are responsible for generating each action potential. These ion channels carry current in the form of ions which move into and out of the cell. Over the past decades, many studies have shown that blockade of these cardiac ion channels can lead to a variety of arrhythmias. Therefore, the most direct method of evaluating the cardiac safety of a compound is to measure its effects on cardiac ion channels.

The cardiac action potential (AP). The AP is classically divided into phases (0-4). Surrounding this AP are the ion currents which are responsible for the different phases. Ion currents which are outward (upward direction) repolarize the cell or return it to a rested state, whereas, ion currents which are inward (downward in direction) depolarize the cell or excite the cell.

Illustration of a transected ion channel in a cell membrane. Ion channels are membrane bound proteins which allow the passage of ions into and out of the cell. Blockade of the ion channel by a drug can interrupt the passage of these ions and cause electrical disturbances in the heart.
CARDIAC ION CHANNEL ASSAYS

| PACKAGES: | Individual ion channel evaluations  
| | CiPA ion channel panel  
| | Chronic exposure /channel trafficking  
| | Characterization of the mechanism of QRS, PR, QT interval prolongation |

| STUDY: | Manual or automated patch clamp  
| | GLP or non-GLP  
| | Recording conditions:  
| | As close to physiologic as possible (temperature, voltage waveforms, ion concentrations) |

| CELL TYPE: | Expression systems  
| | acutely isolated human atrial or ventricular myocytes  
| | iPS-cardiomyocytes |

| TURNAROUND: | 2-5 days from receipt to draft report (depending on assay type and compound number) |

More information on human cardiac ion channels see:
ION CHANNEL BLOCK CAN LEAD TO DIFFERENT ARRHYTHMIAS

**BLOCK OF POTASSIUM CURRENTS**
can lead to QT prolongation and torsade de pointes

**BLOCK OF SODIUM CURRENT ($I_{Na}$)**
can lead to ventricular tachycardia

**BLOCK OF CALCIUM CURRENT ($I_{Ca}$)**
can lead to AV block and negative inotropy.
Providing ion channel data for the FDA and the CiPA initiative


An evaluation of 30 clinical drugs against the comprehensive in vitro proarrhythmia assay (CiPA) proposed ion channel panel.

Crumb WJ Jr, Vicente I, Johannesen L, Strauss DG.

<table>
<thead>
<tr>
<th>Channel Assay</th>
<th>CiPA</th>
<th>P2rx7</th>
</tr>
</thead>
<tbody>
<tr>
<td>hERG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na,1.5</td>
<td>K,7.5</td>
<td></td>
</tr>
<tr>
<td>K,7.1/minK</td>
<td>K,7.5/ K,7.3</td>
<td></td>
</tr>
<tr>
<td>K,4.3</td>
<td>CiPA</td>
<td>K,1.1</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>K,1.2</td>
<td></td>
</tr>
<tr>
<td>Ca,1.2</td>
<td>K,1.4</td>
<td></td>
</tr>
<tr>
<td>Na,1.4</td>
<td>K,1.6</td>
<td></td>
</tr>
<tr>
<td>Na,1.6</td>
<td>K,2.1</td>
<td></td>
</tr>
<tr>
<td>Na,1.7</td>
<td>nAChRa7</td>
<td></td>
</tr>
<tr>
<td>K,1.3</td>
<td>Gaba alpha 1, beta 3, gamma 2</td>
<td></td>
</tr>
<tr>
<td>K,1.5</td>
<td>Gaba alpha 5, beta 3, gamma 2</td>
<td></td>
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<td>hEAGl</td>
<td>Gaba alpha 3, beta 3, gamma 2</td>
<td></td>
</tr>
<tr>
<td>TRAAK</td>
<td>Glu flop</td>
<td></td>
</tr>
<tr>
<td>Stim/Orai</td>
<td>GluR1flop / Aequorin</td>
<td></td>
</tr>
<tr>
<td>Gaba alpha 2, beta 3, gamma 2</td>
<td>GluR1 / GluR2 Flip / Aequorin</td>
<td></td>
</tr>
<tr>
<td>TRPV1</td>
<td>GluR5 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>ASICt</td>
<td>GluR6</td>
<td></td>
</tr>
<tr>
<td>HL-1 cells</td>
<td>GluR6 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>Neuro2A cells (piezo)</td>
<td>GluR6 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>PC12 cells (piezo)</td>
<td>GluR5 / GluR6 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>Ins1 cells (glucose sensitive potassium channels)</td>
<td>GluR5 / KA-2 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>GluR6 / KA-2 / Aequorin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TE671 (endogenous nAch-R with alphal, betal, gamma, delta)</td>
<td>GluR5 / KA-1 / Aequorin</td>
<td></td>
</tr>
<tr>
<td>GluR6 / KA-1/Aequorin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Native Cardiac Ion Channel Assays

<table>
<thead>
<tr>
<th>Role:</th>
<th>$I_{\text{Na}}$ (sodium current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>is responsible for conduction in the heart.</td>
<td></td>
</tr>
<tr>
<td><strong>Cell type:</strong></td>
<td>Human atrial or ventricular myocytes</td>
</tr>
<tr>
<td><strong>Temperature:</strong></td>
<td>23-25°C ($I_{\text{Na}}$ cannot be reliably recorded at physiologic temperatures)</td>
</tr>
<tr>
<td><strong>Positive control:</strong></td>
<td>TTX</td>
</tr>
<tr>
<td><strong>HT:</strong></td>
<td>for screening purposes</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>3-4 concentrations (n=2)</td>
</tr>
<tr>
<td><strong>Manual:</strong></td>
<td>for a detailed analysis</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
</tr>
<tr>
<td><strong>GLP:</strong></td>
<td>for regulatory submission</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Role:</th>
<th>$I_{\text{Ca}}$ (calcium current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>plays an important role in the contraction of the heart</td>
<td></td>
</tr>
<tr>
<td><strong>Cell type:</strong></td>
<td>Human atrial or ventricular myocytes</td>
</tr>
<tr>
<td><strong>Temperature:</strong></td>
<td>35-37°C</td>
</tr>
<tr>
<td><strong>Positive control:</strong></td>
<td>Nisoldipine</td>
</tr>
<tr>
<td><strong>HT:</strong></td>
<td>for screening purposes</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>3-4 concentrations (n=2)</td>
</tr>
<tr>
<td><strong>Manual:</strong></td>
<td>for a detailed analysis</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
</tr>
<tr>
<td><strong>GLP:</strong></td>
<td>for regulatory submission</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Role:</th>
<th>$I_{\text{Ki}}$ (inwardly rectifying potassium current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>helps set the resting potential of the heart.</td>
<td></td>
</tr>
<tr>
<td><strong>Cell type:</strong></td>
<td>Human atrial or ventricular myocytes</td>
</tr>
<tr>
<td><strong>Temperature:</strong></td>
<td>35-37°C</td>
</tr>
<tr>
<td><strong>Positive control:</strong></td>
<td>Ba$^{2+}$</td>
</tr>
<tr>
<td><strong>HT:</strong></td>
<td>for screening purposes</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>3-4 concentrations (n=2)</td>
</tr>
<tr>
<td><strong>Manual:</strong></td>
<td>for a detailed analysis</td>
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<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
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<tr>
<td><strong>GLP:</strong></td>
<td>for regulatory submission</td>
</tr>
<tr>
<td>$IC_{50}$:</td>
<td>6 concentrations (n=5)</td>
</tr>
</tbody>
</table>
NATIVE CARDIAC ION CHANNEL ASSAYS

IRole: $I_{to}$ (transient outward current)
is a repolarizing current
Cell type: Human atrial or ventricular myocytes
Temperature: 35-37°C
Positive control: 4-AP

HT: for screening purposes
$IC_{50}$: 3-4 concentrations ($n=2$)
Manual: for a detailed analysis
$IC_{50}$: 6 concentrations ($n=5$)
GLP: for regulatory submission
$IC_{50}$: 6 concentrations ($n=5$)

IRole: $I_{sus}$ (sustained potassium current)
is a repolarizing current
Cell type: Human atrial or ventricular myocytes
Temperature: 35-37°C
Positive control: 4-AP

HT: for screening purposes
$IC_{50}$: 3-4 concentrations ($n=2$)
Manual: for a detailed analysis
$IC_{50}$: 6 concentrations ($n=5$)
GLP: for regulatory submission
$IC_{50}$: 6 concentrations ($n=5$)

First to provide mechanism for QT prolonging effects of terfenadine in adult human cardiac myocytes
WHY WE USE NATIVE HUMAN CARDIAC MYOCYTES

The importance on proper ion channel physiology and pharmacology of accessory subunits, and a native intracellular milieu with all of the appropriate intracellular messengers has been well described. That is why at CytoBioscience, ion currents can be recorded from acutely isolated human atrial or ventricular myocytes.

Isolated human cardiac myocyte assays
- Most relevant species
- Avoids phenotype issues of iPS cells
- No animals used for tissue harvesting
- Proven experience since 1996

There can be dramatic species differences in cardiac ion channel pharmacology.

This figure illustrates the effect of a compound on $I_{Na}$ recorded from a human cardiac myocyte, a guinea pig myocyte, and a canine myocyte. No species accurately duplicated the effect seen in the human cardiac myocyte.

See also:
European Heart Journal Supplements (2001) 3 (Supplement K), K53–K63
Native and cloned ion channels from human heart: laboratory models for evaluating the cardiac safety of new drugs
I. Cavero1 and W. Crumb2
CARDIAC ACTION POTENTIAL

The cardiac action potential (AP) is a deflection of voltage, lasting several hundred milliseconds. The AP is classically divided into phases (0-4). Phase 0 or the upstroke of the action potential is due to the rapid influx of sodium through the voltage gated sodium channel ($I_{Na}$). This is followed by phase 1, or the rapid phase of repolarization, which is the result of potassium ions leaving the cell through the transient outward potassium current ($I_{to}$). Next is the plateau of the AP, or phase 2, which is in part due to an influx of calcium ($I_{Ca}$) followed by phase 3, or the main phase of repolarization, which is the result of an efflux of potassium through a variety of potassium channels. Finally the resting phase (phase 4) which is controlled in large part by the potassium channel $I_{K1}$.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CELL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>human</td>
<td>adult atrial, ventricular, purkinje fiber</td>
</tr>
<tr>
<td>human</td>
<td>human iPS ventricular or atrial myocytes</td>
</tr>
<tr>
<td>guinea pig</td>
<td>atrial, ventricular, purkinje fiber</td>
</tr>
<tr>
<td>canine</td>
<td>atrial, ventricular, purkinje fiber</td>
</tr>
<tr>
<td>rabbit</td>
<td>atrial, ventricular, purkinje fiber</td>
</tr>
</tbody>
</table>
LANGENDORFF ISOLATED HEART ASSAY

Our team of scientists offers extensive experience leveraging the Langendorff Isolated Perfused Heart preparation to provide a complementary and comprehensive evaluation of possible cardiac responses and/or liabilities.

The isolated-heart preparation is a cost-effective and GLP-validated rapid assessment assay which can simultaneously evaluate electrophysiological, mechanical, and metabolic cardiac end-points with repeatability and accuracy. Notably, this preparation is extremely effective in providing millisecond accuracy for assessing repolarization liabilities and pro-arrhythmic risks (torsadogenic and/or re-entrant.)

Our experts have access to a large comparative database of positive and negative controls and can characterize the following:

- Assessment of electrophysiological responses and liabilities - particularly chronotropy, dromotropy, and changes in the duration, temporal stability and spatial heterogeneity of ventricular repolarization (via trans-mural/ventricular bipolar electrograms and monophasic action potentials)

- Characterization of direct (load-independent) cardiac functional/mechanical effects (via isovolumetric left ventricular pressures)

- Quantification of myocardial metabolic imbalances/loading and coronary flow/vascular resistance

- Evaluation of dose-response and/or use-dependency

- Establishing of NOEL and NOAEL leveraging our ion-channel experience to provide mechanistic understanding
**CONSCIOUS PREPARATIONS**
- Telemetry (Invasive and Non-Invasive; ECG and systemic/ventricular pressures)
- Cardiac Output and Chronic Load-Independent Cardiac Function
- Imaging (Echocardiography, CT, MRI, x-ray)
- Blood Sampling (PK)

**ANAESTHETIZED PREPARATIONS**

**SURGICAL MODELS AND DEVICE ASSESSMENTS**

**COMPREHENSIVE METHODOLOGIES**
- Complete Physiological Evaluation
- ECG (rhythm, intervals, repolarization, beat-to-beat analyses)
- Systemic and Cardiac Hemodynamics
- Cardiac Output, Fractionation/Regional Distribution, and Blood Flow Load-independent ventricular function and Ventriculo-vascular coupling
- Myocardial/Systemic Energetics and Oxygenation
- Electrophysiology
  - His-bundle
  - programmed-electrical stimulation
  - “mapping”
  - conduction
- Renal (GFR/RPF)
- Baroreceptor/Autonomic
- Respiratory (mechanics)
- Vascular Function Histology

**DISEASE MODELS**
- Heart Failure (chronic and acute)
  - Systolic (embolization, tachy-pacing, coronary-ligation, banding, drug-induced)
  - Diastolic (renoprival hypertension, diabetes, genetic)
- Arrhythmia (e.g. atrial fibrillation, sudden-death, torsades de pointes, reperfusion)
- Ischemia and/or Ischemia/Reperfusion
- Pulmonary hypertension (drug-induced, embolism, hypoxia)
- Systemic hypertension (renoprival, genetic, diet)
- Diabetes
- Embolism/Atherosclerosis (endothelial injury, diet/drug-induced, Foltz)
- Hemorrhagic shock
- Pain (Cold-pressor)

**GLP compliant studies for IND submission**

**Non-GLP**

**Multiple Species: small (e.g., mice, rat, GP) to large (e.g., dog, sheep, pig, non human primate)**
One of the aims of the CiPA initiative is to integrate data from patch clamp experiments on a range of important ion channels with *in-silico* simulations of cardiac electrical activity. Currently the O’Hara & Rudy model (see figure 1) is a hot candidate to be the model of choice, since it is the only available model of human ventricular myocytes based entirely on human data.

Due to their complexity, effective use of these models and interpretation of results requires extensive experience in the field. Here at CytoBioscience we are ready to support our customers both in *in-vitro* electrophysiology as well as in *in-silico* modeling. With our simulations we can help to make sense of your data.

**SERVICES OFFERED**

- General advice on *in-silico* modeling of cardiac electrical activity
- Implementation of a range of available cellular models
- Disease state modeling (e.g. hypertrophic cardiomyopathy, ischemia, long-QT syndromes). See figure 1 for an example.
- Implementing ion channel block based on electrophysiology data, plotting and interpretation of results

*Figure 1: Example simulation of the O’Hara & Rudy 2011 ventricular model in endocardial configuration in normal conditions and under hypertrophic cardiomyopathy conditions. Application of 2nM Dofetilide was simulated by blocking 59% of $I_{Kr}$ and 17.4% of $I_{to}$. The physiological model action potentials were prolonged (black traces), but no arrhythmogenic activity was observed. In hypertrophic cardiomyopathy (red traces), the same percentage block of $I_{Kr}$ and $I_{to}$ causes alternans and early-after-depolarizations.*
Adhering to Global Regulatory Guidelines, the CytoBioscience Analytic Services Department offers capabilities to develop, qualify, and validate analytical methods in the support of non-clinical dose formulation analyses for all in-vivo, ex-vivo, or in-vitro platforms. Specific examples include discovery, safety pharmacology, toxicology, genetic toxicology, and ion channel studies. We offer personalized attention, fast analysis, and quality data and reports to provide the results that are needed to meet program needs and deadlines.

**ANALYTICAL SERVICES**

**CYTOBIOSCIENCE OFFERS:**

- **Flexible scheduling**
- **Rapid study starts**
- **Personalized attention**
- **Strict adherence to regulatory guidelines**
- **HPLC method development with:** method validation/qualification, assessing specificity, selectivity, accuracy, precision, and stability
- **HPLC analysis** for the determination of concentration and homogeneity in non-clinical dose formulations
- **HPLC analysis** for determination of purity
YOU’VE GOT QUESTIONS?
WE’VE GOT ANSWERS.

You’d like to learn more about CytoBioScience, our focus and our work? We have a dedicated team of well-trained and high-professionalized specialists located in our offices to meet our customers’ needs. We’re looking forward to talking to you.

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