



Cardiac Safety Screening Service Against Multiple Ion Channel Targets

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I. Background

Many new pharmaceutical agents have been withdrawn from the market, or severely restricted to specific indications, due to adverse events. Furthermore, these compounds had diverse chemical structures and are used in different therapeutic areas. Cardiac, hepatic, and hematological abnormalities are the major causes of such withdrawals. The effect of new drugs on ventricular repolarization, specifically its prolongation, is now the most common cause of drug withdrawals and delays in regulatory approval for marketing. For these reasons, recent regulatory developments have thrust cardiac safety to the forefront of clinical development.

Non-clinical safety pharmacology merges the disciplines of toxicology and pharmacology. Both the ICH S7A and the latest ICH S7B guidelines have recognized the importance of safety pharmacology testing. Assessing the potential for adverse effects on vital systems including the cardiovascular, respiratory, and central nervous system is critical in the drug development process. Today, the pharmaceutical industry must comply with many regulatory guidelines; thus, it is facing numerous challenges. For instance, there is a lack of clarity as to the type and extent of pre-clinical testing is required. However, Aurora Biomed Inc. has compiled a series of protocols to assess these unmet needs.

II. Introduction

In the past few years, numerous drugs have been withdrawn from the market due to their interaction with certain ion channels. These interactions create adverse cardiac side effects that may lead to acquired Long QT syndrome (LQTS)¹. Such drugs induce LQTS predominantly by modulating the human ether-à-go-go-related gene (hERG) channel. The hERG potassium channel underlies IKr repolarization currents of the cardiac action potential. Detecting hazardous side effects on hERG channel too late in the process of drug development will dramatically increase the duration, and consequently the cost, of drug development². Screening compounds for hERG liability early in the drug development process is likely to cut costs. Moreover, other ion channels (SCN5A, KCNQ1 and Kv1.5) thought to be involved in cardiac safety have recently surfaced in the literature.

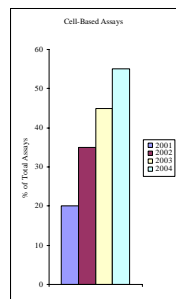
The ICH S7A and the more recent ICH S7B guidelines have outlined the following aspects that need to be considered while carrying safety screening of compounds:

- Screening should be scientifically based
- More than one type of assay should be used
- Multiple ion channels may be involved
- Multicellular preparations of stable model systems
- Expression systems should be carefully selected
- Ion channel variation among species
- Appropriate drug concentrations should be used
- Drug ADMET aspects should be respected
- Reliability of high throughput in vitro screening assays should be maintained

Aurora Biomed Inc. understands these requirements; furthermore, it takes into account the future of cell based assays (Figure 1) in order to address the needs of the research community.

Figure 1. The future of cell based assays

- More than 55% of HTS assays by 2005
 - MTS > 100,000 dp/week
 - HTS > 100,000 dp/week
- Provide
 - Functional read outs
 - Permeability & cytotoxicity info
 - Better prediction of compound behavior



Box 2004: Drug Discov World 5:21-30

III. Materials & Methods

A. hERG Screening Protocol

Aurora Biomed Inc. uses a CHO-hERG cell line and cold rubidium flux assay method to screen compound libraries for potential hERG blockage. The Rb⁺ ion is used as tracer for the K⁺ ion. Typical compounds such as Astemizole, Pimozide, E-4031, Terfenadine etc. are used as positive controls of hERG to validate the assay.

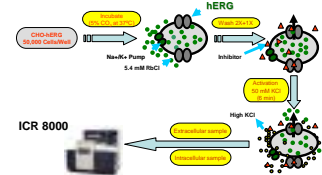


Figure 2. Cells are incubated in cold Rubidium (Rb⁺) for 30 minutes, washed, and then activated using a high concentration of KCl. The supernatant is removed and cells are lysed. Both the extracellular and intracellular samples containing Rb⁺ are analyzed on the ICR. The compounds of interest are added in the 3rd wash as well as in the activation buffer.

IV. Results

B. IC₅₀ Curves for Standard hERG Blockers

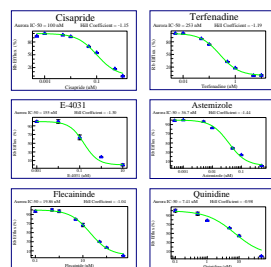


Figure 3. Dose response curves for Astemizole (a), Cisapride (b), E-4031 (c), Terfenadine (d), Flecainide (e) and Quinidine (f). The % Rb⁺ efflux was calculated as (Rb_{supernatant}/Rb_{total}) x 100. The X-axis is standardized and the Hill Coefficient shown (n=4).

C. Correlation of Flux Assay with Electrophysiology

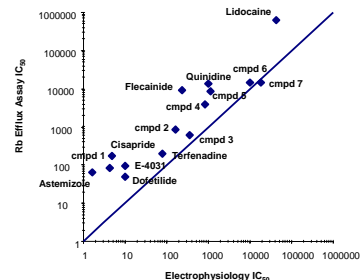
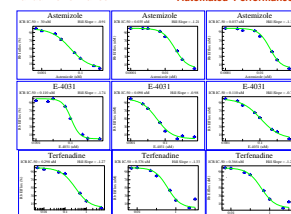


Figure 4. Comparison of potency estimates for hERG blockage between patch clamp and ICR flux assay IC₅₀ values. Patch clamp potency estimates were obtained using the peak tail current obtained at -50 mV following a 4 second depolarization to +20 mV in escalating concentration of the drug. The thick diagonal line represents the line of unity. The correlation coefficient for a linear fit to the data is 0.88, which suggests a good fit.

D. hERG Screening with Automated Flux Assay



Manual Performance vs. Automated Performance



Drug	PERFORMANCE	
	Manual	Automated
Astemizole	30	37
E-4031	110	110
Terfenadine	258	376

Figure 5. Dose response curves for standard hERG blockers (Astemizole, E-4031 and Terfenadine) obtained with the Automated Assay System. The IC₅₀ values obtained with the automatic system are comparable to those found manually. The adjacent image shows the Automated Assay System (CO₂ incubator, liquid handler, plate stacker and washer) coupled with an ICR-12000.

E. Multiple dose Screening Showing % Efflux of Test Compounds, Activated and Basal Efflux in the Automated Assay

Drug	Basal Efflux	Activated Efflux	Basal Efflux	Activated Efflux
10	0.00	1.74	2.03	0.06
100	0.00	1.26	1.03	1.51
1000	0.00	0.00	1.03	0.06
10000	0.00	0.78	1.03	0.07
100000	0.00	0.00	0.00	2.00
1000000	0.00	0.00	1.06	0.00
Automated Efflux	1.07			
Basal Efflux	2.06			

F. Multiple Dose Screening Showing Acceptable Standard Deviation Encountered in the Automated Assay

Drug	Basal Efflux	Activated Efflux	Basal Efflux	Activated Efflux
10	0.00	1.74	2.03	0.06
100	0.00	1.26	1.03	1.51
1000	0.00	0.00	1.03	0.06
10000	0.00	0.78	1.03	0.07
100000	0.00	0.00	0.00	2.00
1000000	0.00	0.00	1.06	0.00
Automated Efflux	1.07			
Basal Efflux	2.06			

V. Important Information

What we can provide:

- Screening of compounds for hERG safety
- Assay development and optimization
- Analysis of assay samples on the Ion Channel Reader

When you can get the results:

- For analyzing the assay samples on ICR 1-2 days + shipping time
 - For screening compounds 2-3* days + shipping time
 - Assay development/optimization 30-60 days + shipping time
- *(dependent upon the number of samples)

What is the cost of the hERG safety screening?

Pricing details can be obtained from our sales representatives.

VI. Future Directions

Aurora Biomed Inc plans to provide single/ multiple dose screening against the following cardiac ion channel targets:

- hERG
- SCN5A
- KCNQ1
- KV1.5

VII. Conclusion

hERG is an important ion channel involved in cardiac safety screening. Aurora Biomed Inc.'s hERG screen service is robust, reliable, sensitive and fast. It is planned to address multiple ion channel targets of cardiac safety.

VIII. References

1. Keating M T, Sangunetti M C (2001) Cell. 2001 Feb 23;104(4):569-80.
2. Netzer R. et al. (2001) Drug Discovery Today; 6(2):78-84.
3. Terstappen G. C. (1999) Analytical Biochemistry, 272, 149-155.
4. Sikander Gill et al. (2003) Drug Discovery, ASSAY and Drug Development Technologies 1(5):709-717.