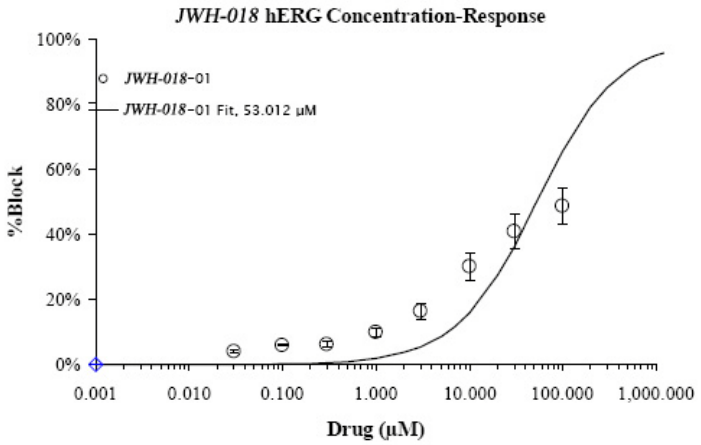
hERG: *In vitro* Test For The Potential of QT Interval Prolongation by JWH-018

hERG FastPatch Individual Data:

Test Article	IC50 (μM)	Conc (μM)	Mean % hERG Inhibition	Standard Deviation	Standard Error	n	Individual Data (% Inhibition)
JWH-018	>100	0.03	4.09	0.0	0.0	2	4.6
							3.5
		0.1	6.04	0.0	0.0	2	6.3
							5.8
		0.3	6.23	0.0	0.0	2	7.1
							5.4
		1	9.88	0.0	0.0	2	11.2
							8.6
		3	16.34	0.0	0.0	2	18.6
							14.1
		10	30.13	0.1	0.0	2	34.4
							25.8
		30	40.95	0.1	0.1	2	46.3
							35.6
		100	48.74	0.1	0.1	2	54.4
							43.1



hERG Background:

"Test substance concentrations for *in vitro* studies should span a broad range, covering and exceeding the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration-limiting. Ideally, the duration of exposure should be sufficient to obtain steady-state electrophysiological effects, unless precluded by the viability of the cell or tissue preparation. The duration of exposure should be indicated. Appropriate positive control substances should be used to establish the sensitivity of the *in vitro* assay system as well as to confirm that the ion channels of interest are present and stable."

"In vitro electrophysiology studies can provide valuable information concerning the effect of a test substance on action potential duration and/or cardiac ionic currents. These assays have an important role in assessing the potential for QT interval prolongation and elucidating cellular mechanisms affecting repolarization. *In vitro* electrophysiology studies employ either single cell (e.g., heterologous expression systems, disaggregated cardiomyocytes) or multicellular (e.g., Purkinje fiber; papillary muscle; trabeculae; perfused myocardium; intact heart) preparations. Multicellular preparations are stable test systems to study action potential duration. While more fragile, single cell preparations minimize diffusional barriers to the site of action. The analysis of parameters for each phase of the action potential such as Vmax for phase 0 (INa), APD30 for phase 2 (ICa) and "triangulation" for phase 3 (IK) can be useful to investigate the effects on specific channels responsible for these phases. In addition, some parameters derived from the Langendorff preparation have been reported to

provide information regarding proarrhythmia. Heterologous expression systems, where human ion channel protein(s) are expressed in noncardiac cell lines, are used to assess the effects of a test substance on a specific ion channel. Disaggregated myocytes are technically more challenging than the expression systems but have the advantage of being suitable for assessing effects on both action potential duration and ionic currents."

Standard Methods:

hERG Fast Patch Cell culture: HEK293 cells were stably transfected with hERG cDNA. Stable transfectants have been selected by coexpression with the G418-resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including G418 in the culture medium. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/ F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate and 500 µg/mL G418. Before testing, cells in culture dishes were washed twice with Hank's Balanced Salt Solution, treated with trypsin and re-suspended in the culture media (1-1.5 x106 cells in 20 mL). Cells in suspension were allowed to recover for 1-3 hours in a tissue culture incubator set at 37°C in a humidified 95% air, 5% CO2 atmosphere. Immediately before use in the PatchXpressR system, the cells were washed in HB-PS to remove the culture medium and re-suspended in 150 µL of HB-PS.

Test Method. All experiments were performed at ambient temperature. Each cell acted as its own control. Test Article Treatment Groups. Two concentrations were applied at five (5) minute intervals via disposable polyethylene micropipette tips to cells expressing hERG ($n \geq 2$,where n = the number cells/concentration). Each solution exchange, performed in quadruplicate, consisted of aspiration and replacement of 45 µL of the total 50 µL volume of the extracellular well of the Sealchip16. Duration of exposure to each test article concentration was five (5) minutes.

Test Article Application Schedule Solution	Procedure	Exposure time
Vehicle control	four 45 µL exchanges	10 min
Test article concentration 1	four 45 µL exchanges	5 min
Test article concentration 2	four 45 µL exchanges	5 min

Positive Control Treatment Group. Vehicle was applied to cells expressing hERG ($n \geq 2$, where n = the number cells), for a 10-minute exposure interval. Each solution exchange, performed in quadruplicate, consisted of aspiration and replacement of 45 µL of the total 50 µL volume of the extracellular well of the Sealchip16. After vehicle application, the positive control was applied in the same manner, to verify sensitivity to hERG blockade. Automated Patch Clamp Electrophysiological Procedures. Intracellular solution for whole cell recordings consisted of (composition in mM): potassium aspartate, 130; MgCl2, 5; EGTA, 5; ATP, 4; HEPES, 10; pH adjusted to 7.2 with KOH. This solution was prepared in batches, aliquoted, stored frozen, and a fresh aliquot thawed each day. In preparation for a recording session, intracellular solution was loaded into the intracellular compartments of the Sealchip16 planar electrode. Cell suspension was pipetted into the extracellular compartments of the Sealchip16 planar electrode. After establishment of a whole-cell configuration, membrane currents were recorded using dual-channel patch clamp amplifiers in the PatchXpressR system. Before digitization, the current records were low-pass filtered at one-fifth of the sampling frequency.Voltage-Clamp Procedures. Onset and block of hERG current was measured using a stimulus voltage pattern (Figure 1, lower panel) consisting of a 500 ms prepulse to –40 mV (leakage subtraction), a 2-second activating pulse to +40 mV, followed by a 2-second test pulse to -40 mV. The pulse pattern was repeated continuously at 10 s intervals, from a holding potential of -80 mV. Peak tail current (Figure 1, upper panel) was measured during the -40 mV test pulse. Leakage current was calculated from the current amplitude evoked by the prepulse and subtracted from the total membrane current record.