Inhibition of hERG by siRNA Takes More Than 8 hrs to Manifest: Implications for Designing hERG Assays

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Introduction

Nonclinical safety pharmacology studies for siRNA follow a hybrid of the small molecule (SM) guidance and biologics guidance. The Oligonucleotide Safety Working Group (OSWG) has published a series of recommendation papers for oligonucleotides, including recommendations for safety assessment, because development of oligonucleotide-based therapeutics is not addressed by regulations. This is especially true regarding the hERG assay, which is a core assay in ICH S7B for SM. While OSWG states that a hERG study is not necessary for IND submission, all approved siRNAs have submitted hERG data which are necessary for requesting a TQT waiver. Every hERG study on siRNA candidates was performed using the same protocol (less than 20 min application) as for testing a SM. It has been established that siRNA-mediated gene silencing takes approximately 48-96 hr, indicating that much longer exposure is required for detecting a siRNA-induced effect. To investigate the time-course for siRNA-mediated inhibition of hERG function, commercially available siRNAs, hERG siRNA (positive control) and a scrambled siRNA (negative control) were evaluated in a stable hERG transfected cell line (CHO) by whole-cell voltage clamp using QPatch 48 (single hole). The hERG siRNA (Horizon Discovery, Cambridge, UK) was composed of 4 siRNAs which targets four different mRNA regions of hERG located at C-linkers and S5 side of the P-loop. In the acute study, 100 nM siRNAs were applied as 12 additions over a 20-minute period, no significant effects were observed by either siRNAs. The chronic effects of 100 nM siRNAs on hERG were evaluated by transfecting the CHO-hERG cell lines. The current amplitudes were recorded in QPatch at 8, 16, 24, and 48 hr after transfection. At 8 hr there was no significant difference in the current amplitudes between the hERG siRNA and scrambled siRNA. At 16 hr, reduction of current amplitudes by hERG siRNA was detected compared to scrambled siRNA (↓63%), which became 81% at 48 hr. These results suggest that short term (20 min) exposure in the hERG assay is insufficient to detect an effect with a hERG siRNA, which mechanism requires much longer application. Specific and appropriate guidance for oligonucleotides should be developed based on their characteristics.

Methods

- *The hERG siRNA*

The hERG siRNAs (ON-TARGETplus Human KCNH2 siRNA SMARTPool Catalogue ID: L-006233-00-0050) were ordered from Horizon Discovery (Dharmacon), which is a mixture of 4 siRNAs as shown in the following. Negative siRNA (Horizon Discovery) was used as control, which are called ON-TARGETplus Non-targeting Control Pool at Horizon Discovery (Catalog ID: D-001810-01-50; D-001810-02-50; D-001810-03-50; D-001810-04-50). These 4 siRNAs are described as negative siRNA #1 to #4.

Homo sapiens KCNH2 transcript variant 1 mRNA (NM_000238.4)

- siRNA 1 targeting location: 2015-2033: CGCGGAAGCUGGAUCGCUA; c-linker region
- siRNA 2 targeting location: 1844-1862: ACGAGGAGGUGGUCAGCCA; between S5P and P
- siRNA 3 targeting location: 2175-2193: GGGCGACCAGAUAGGCAAA: c-linker region
- siRNA 4 targeting location: 2140-2158: CACAUGGACUCACGCAUCG: c-linker region

- *The hERG Assay*

The hERG current was recorded by automatic whole-cell voltage-clamp technology, QPatch 48 (Sophion, Denmark) in a CHO cell line. A standardized protocol was used to elicit ionic current through the hERG potassium channel. Cells were held at -80 mV. Membrane potential was first depolarized to –50 mV for 0.5 s. A depolarization to +30 mV for 2 s was used to activate hERG channel followed by a repolarization to -50 mV for 2 s to elicit a tail current. This stimulation paradigm repeated once every 10 s (0.1 Hz). All electrophysiology experiments were performed at room temperature (18 – 22° C).

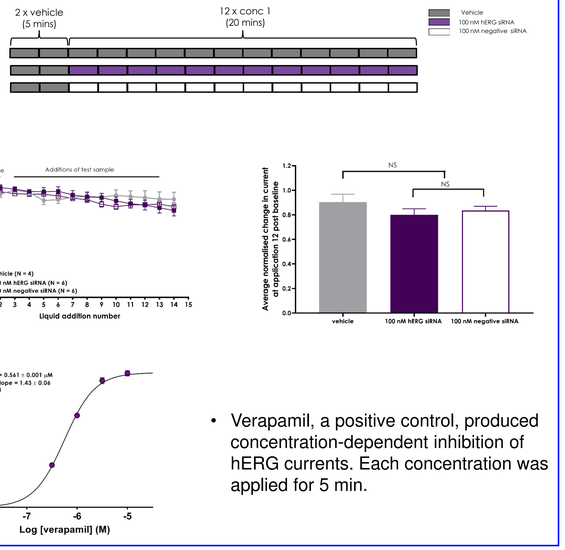
- *Acute hERG Assay*

The effects of one concentration (100 nM) of two siRNA molecules, one positive and the other one negative, were evaluated on hERG current. The siRNA molecules were applied as twelve additions over a 20-minute period.

- *Transfection of siRNA in CHO-hERG cells*

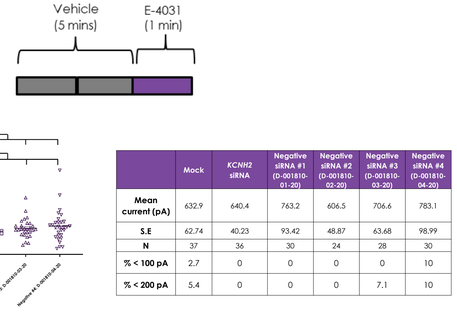
DharmaFECT (Horizon Discovery) has been selected for transfection as this is the proprietary transfection reagent and method provided by the manufacturer (Horizon Discovery) of the siRNA molecules. DharmaFECT 4 Transfection Reagent (Cat No: T-2004-03) was used because it is recommended for CHO-K1 cells.

Figure 1. Acute hERG Assay: 20 min Application of siRNA Has No Effects on hERG Currents



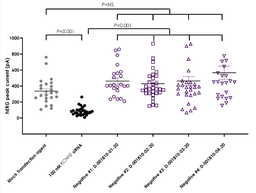
- Verapamil, a positive control, produced concentration-dependent inhibition of hERG currents. Each concentration was applied for 5 min.

Figure 2. Transfection of siRNA for 8 hrs Has No Effects on hERG Currents



	Mock	*KCNH2* siRNA	Negative siRNA #1 (D-001810-01-20)	Negative siRNA #2 (D-001810-02-20)	Negative siRNA #3 (D-001810-03-20)	Negative siRNA #4 (D-001810-04-20)
Mean current (pA)	632.9	640.4	763.2	606.5	706.6	783.1
S.E	62.74	40.23	93.42	48.87	63.68	98.99
N	37	36	30	24	28	30
% < 100 pA	2.7	0	0	0	0	10
% < 200 pA	5.4	0	0	0	7.1	10

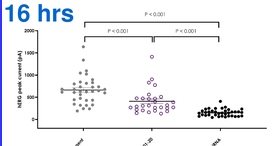
Figure 3. Transfection of siRNA for 48 hrs Induced Significant Reduction of hERG Currents



	Mock	*KCNH2* siRNA	Negative siRNA #1 (D-001810-01-20)	Negative siRNA #2 (D-001810-02-20)	Negative siRNA #3 (D-001810-03-20)	Negative siRNA #4 (D-001810-04-20)
Mean current (pA)	337	86.43	462.1	429.6	463.3	566.4
S.E	32.42	10.46	39.46	37.36	56.19	81.22
N	24	27	23	32	30	24
% < 100 pA	0	74.1	0	0	6.7	0
% < 200 pA	16.7	96.3	0	9.4	16.7	8.3

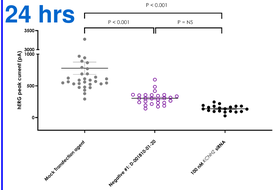
Figure 4. Transfection of siRNA for 16 or 24 hrs Induced Reduction of hERG Currents

16 hrs



	Mock	*KCNH2* siRNA	Negative siRNA #1 (D-001810-01-20)
Mean current (pA)	664.2	153.0	410.0
S.E	52.5	12.8	53.1
N	35	37	28
% < 100 pA	0	29.8	0
% < 200 pA	2.9	78.4	17.9

24 hrs



	Mock	*KCNH2* siRNA	Negative siRNA #1 (D-001810-01-20)
Mean current (pA)	777.9	139.6	304.0
S.E	97	11.7	19.4
N	33	20	26
% < 100 pA	0	30	0
% < 200 pA	0	90	15.4

Figure 5. Time-dependent Effects of hERG siRNA Tested at 100 nM

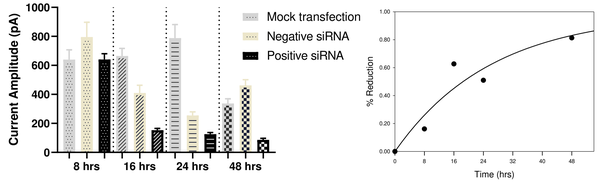
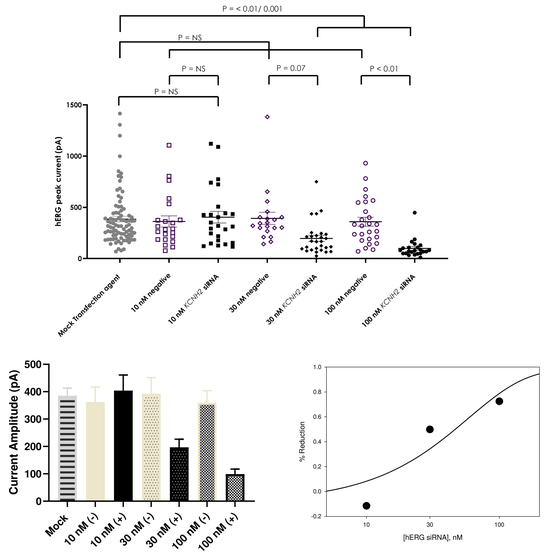


Figure 6. Concentration-dependent Effects of siRNA Tested at 48 hrs



Summary

1. **Acute siRNA study**
   - siRNA has no effects on hERG during 20 min application
   - siRNA should not be treated as a SM in the hERG assay
     - False negative
2. **Chronic siRNA study**
   - At 8 hrs after transfection, siRNA had no significant effects on hERG function
   - At 48 hrs after transfection, siRNA had significant effects on hERG function
   - At 16 & 24 hrs only, the negative siRNA mediated hERG inhibition
     - Mechanism unknown, e.g., off-target binding or loss of cellular integrity
   - At 48 hrs, siRNA inhibited hERG function in a concentration-dependence manner

Recommendation

- **Novel siRNA should be tested in hERG assay at 48 hrs after transfection based on its mechanism of action**
  - A negative siRNA should be included in addition to mock transfection
  - Acute application is not needed