Discussion Points for Panel Discussion

In vitro cardiac electrophysiological assay (hERG assays)

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  - ***General considerations***
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Mentioned in Guideline S6

- **S6: PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS**
  - It is important to investigate the potential for undesirable pharmacological activity, and to incorporate particular monitoring for these activities in the toxicity studies and/or clinical studies.
  - **Investigations may also include the use of isolated organs or other test systems not involving intact animals.**
  - All of these studies may allow for **a mechanistically-based explanation of specific organ toxicities**, which should be considered carefully with respect to human use and indication(s).

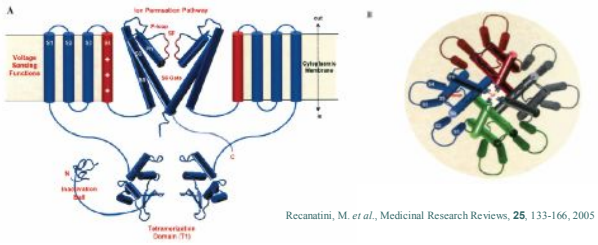
Mentioned in Guideline S7A

- **S7A: SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS**
  - For biotechnology-derived products that **achieve highly specific receptor targeting**, it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies, and therefore **safety pharmacology studies can be reduced or eliminated** for these products.
  - For biotechnology-derived products that represent a novel therapeutic class and/or those products that **do not achieve highly specific receptor targeting, a more extensive evaluation by safety pharmacology studies should be considered.**

Mentioned in Guideline S7B

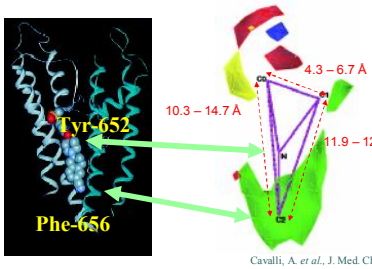
- **S7B: THE NON-CLINICAL EVALUATION OF THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS**
  - This guideline **applies to new chemical entities for human use** and marketed pharmaceuticals when appropriate (e.g., when adverse clinical events, a new patient population, or a new route of administration raises concerns not previously addressed).

Molecular structure of the hERG channel



Recanatini, M. *et al.*, Medicinal Research Reviews, **25**, 133-166, 2005

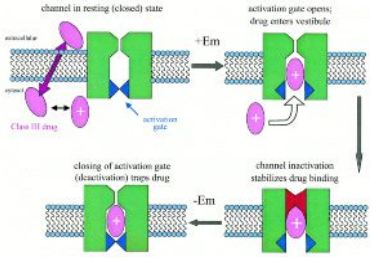
Binding site structure of the hERG channel



Cavalli, A. *et al.*, J. Med. Chem., **45**, 3844-3853, 2002

The drug binding site of the hERG channel is located in the central cavity of the channel and should be inaccessible to large molecules such as therapeutic protein biologics

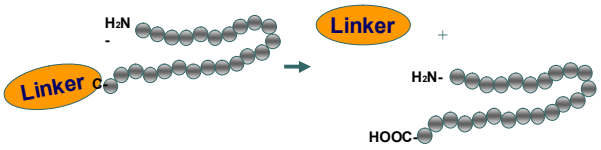
Inhibition mechanism of chemical drugs in the K channel



Tristani-Firouzi, M. *et al.*, Am. J. Med., **110**, 50-59, 2001

Because protein biologics have limited intracellular penetration, they would not be expected to reach the binding site typical of a hERG blocker.

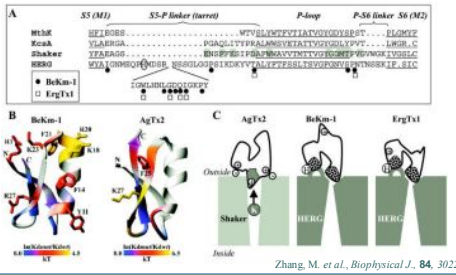
Case 1: Bioconjugates with organic linkers



Detached linkers may act on the hERG channel. Therefore, the following points should be considered.

- **Size of the linker.**
- **Whether or not the linker is a new entity.**

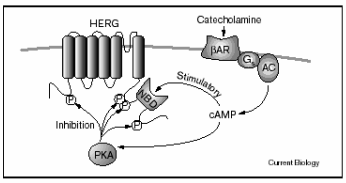
Case 2: Toxin binding site



Zhang, M. *et al.*, *Biophysical J.*, **84**, *3022-3036*, 2003

The peptide toxin binding site of the hERG channel is located external to the channel, but most protein biologics are unlikely to bind with such a specific site.

Case 3: Receptor-mediated signal transduction



A model illustrating the complexity of cAMP regulation of HERG K+ channel protein using two distinct pathways. Stress elevates catecholamines that stimulate β-adrenergic (βAR) receptors to activate, via G-protein (G_s), adenyl cyclase (AC). The resulting increase in cAMP produces regulation of HERG. NBD, nucleotide-binding domain.

Cui, J. *et al.*, *Curr. Biol.*, **10**, 671-674, 2000

The functional effects of channel accessory subunits, receptors, kinases and phosphates may modify channel expression, trafficking, or channel activity.

Case 4: Tumor necrosis factor (TNF)- α

- TNF- α is a potent inducible cytokine consisting of 157 amino acids which are mainly produced by monocytes.
- TNF- α consistently and reversibly decreased hERG current via activation of TNF receptor I.
- A superoxide dismutase prevented depression of TNF- α -induced hERG current.
- TNF- α system impairs hERG function mainly by stimulating superoxide anion, not by altering hERG expression.

Wang, J. *et al.*, *J. Biological Chem.*, **279**, *13289-13292*, 2004

hERG channel may be affected by a secondary effect, not by direct blocking of the channel or expression.

Summary on hERG assays

- ***General considerations concerning bioconjugates***
  - **Not likely to enter cells and block channel as do small molecules (size matters).**
  - **Not likely to interact with other proteins except for highly specific receptor targets.**
- ***Feasibility***
  - **Effects of an organic linker in a bioconjugate on the hERG channel.**
  - **Binding to the toxin binding site.**
  - **Secondary effects, not a direct reaction to hERG channel.**
- ***Conclusions***
  - **For biopharmaceuticals, *in vivo* test systems are more appropriate and better predictors of QT prolongation risk than *in vitro* assays.**
  - **If QT prolongation is observed *in vivo*, the mechanism should be clarified using an *in vitro* system.**