Safety Pharmacology Evaluation of Biologics

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Outline of Presentation

- Guidances
- Specific Requirements
- Special Considerations for Biologics
- Basic Testing Approaches
- Conclusion

Relevant ICH Guidances

- *S6* – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
- *S7A* – Safety Pharmacology Studies for Human Pharmaceuticals
- *S7B* – The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
- *E14* – The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs

Guidance Specifics

- *ICH S6*: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
  - Case-by-case, science-based approach
  - Can investigate in separate studies or incorporate into design of toxicity studies
  - Aim is to reveal functional effects on major physiological systems (eg, CV, CNS, respiratory)

- *ICH S7A*: Safety Pharmacology Studies for Human Pharmaceuticals
  - "..adopt a rational approach…design based on individual properties and intended use…"
  - "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."

Guidance Specifics

- *ICH-S7B*:
  - Extension and complement to ICH-S7A
  - "… applies to NCE for human use and marketed pharmaceuticals when appropriate (eg, when adverse clinical events, a new patient population, or a new route of administration raises concerns not previously addressed)."
  - "Conditions under which studies are not called for are described in ICH-S7A."
  - "***In vitro* and *in vivo*** assays are complementary approaches; therefore, according to current understanding, **both** assay types should be conducted."

Guidance Specifics

- *ICH-E14*:
  - "…generally applicable to new drugs having systemic bioavailability, but may not apply to products with highly localized distribution and those administered topically and not absorbed."
  - "…concerned primarily with the development of novel agents….might also be applicable to approved drugs when a new dose or route of administration is being developed that results in significantly higher exposure."
  - Thorough QT/QTc study expected to be conducted relatively early in clinical development.
  - "Factors that could reduce the need for such a study include the inability to conduct in healthy volunteers or patients, how the drug is studied and used (eg, administered under continuous monitoring), as well as **nonclinical** data."

Safety Pharm Requirements

- Sensitivities around and need for safety pharmacology evaluation consistent between both biologics and small molecules
- While study (assay) methods are generally comparable, approaches taken may vary considerably, particularly for biologics
- Biological activity, scientific rationale, clinical relevance, and study feasibility should all drive program design
- In particular…..

Special Considerations for Biologics

- Nature and size of molecule
  - Monoclonal antibodies (MAbs), fusion proteins, cytokines, hormones, growth factors, enzymes, thrombolytics, etc.
  - MW range ~ 1000 (peptides) to >140,000 (MAbs) Daltons
- Structure
  - Complex, heterogeneous
- Molecular target and expression
  - High specificity and selectivity, potential for exaggerated pharmacology
- Species specificity of molecule
  - Human, NHP?, rodent?
  - Relevant (responsive) testing species
    - Presence of a relevant epitope and biological activity
    - Specificity and affinity appropriate

Molecular Size of Biologics: Size Matters

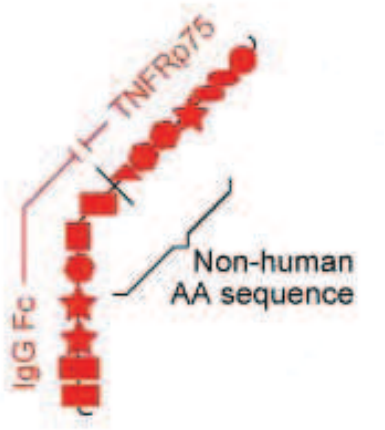
- Typical "Drug-like" molecules: Small
  - Lipinsky, 1997
    - "Drug-like" molecules are lipophilic and have low MW (<500 d)
  - Ghose et al., 1998 (N=6304 drugs)
    - Low MW (≤ 700 d; avg = 357 d)

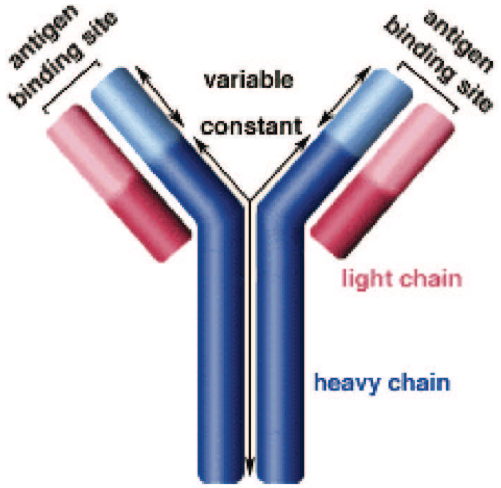
- Biologicals or Protein Thrapeutics: Large
  - Range: 1,000 to >140,000 d
  - Restricted from crossing plasma membrane

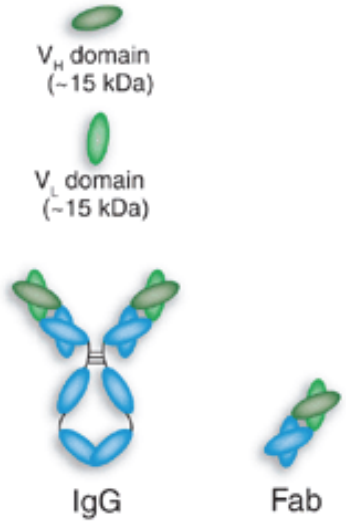
Types of Protein Therapeutics

- Antibodies
  - Polyclonals, monoclonals: chimeric, humanized, fully human
    - Remicade, Rituxan, Xolair, Avastin, Humira, Vectibix, etc.
- Recombinant Proteins
  - Mostly ligands and enzymes that stimulate processes
    - Insulin, EPO, GCSF, GH, GMCSF, Thrombin, t-PA, etc.
  - A few antagonists, e.g., IL-1Ra
- Fusion Proteins
  - Mostly receptor fusion that are antagonists
    - Enbrel, etc.
  - Some peptide fusions, e.g., Nplate
- Peptides
  - Agonists and Antagonists
    - Byetta (exenatide), PTH, etc.

Structure and Characteristics of Various Types of Therapeutic Protein Biologics







Fusion Protein (e.g., Enbrel)
- Typically consists of protein sequence that binds specifically to target, fused with the Fc domain of IgG
- Can be dimeric

Full-length Ab (e.g., Avastin)
- Typically have systemic half-lives in excess of 20 days (due to the binding of Fc domain to cell receptors)
- High molecular weights

Fab (e.g., Lucentis)
- Absence of Fc domain shortens systemic elimination half-life
- Minimizes systemic exposure; more rapid clearance
- Lowers the possibility of cytotoxicity and inflammation
- Lower molecular weight than full-length Ab

Special Considerations for Biologics

- Disposition and biological activity of molecule
  - Proteolytic degradation
  - Distribution
  - Clearance
  - Half-life (<1h for enzymes – 3 weeks for MAbs)
  - Duration of effect
- Immunogenicity
  - Potential development of anti-drug antibodies
- Physicochemical characteristics of molecule
  - Charge, tendency to aggregate, etc
- Formulation components
  - Tween, sucrose, etc

Clinical Adverse Reactions Associated with Biologics

- Related to pharmacology
- Driven by target activity

Rx/Indication	Target	Intended Effect	Adverse Event
bevacizumab (oncology)	Anti-VEGF	Anti-angiogenesis	Poor wound healing
Infliximab (RA; Crohns)	Anti-TNFα	Immuno-suppressive	Opportunistic infections
trastuzumab (oncology)	Anti-HER2 (ERBB2)	Anti-proliferative + ADCC	Congestive Heart Failure

Basic Safety PharmacologyTesting Approaches

- Hierarchy of Organ Systems Acutely Critical for Sustaining Life (First Tier)
  - Cardiovascular System
  - Central Nervous System
  - Respiratory System

- Second Tier Organ Systems (of less immediate investigative concern)
  - Gastrointestinal System
  - Renal System
  - Immune System
  - Other?

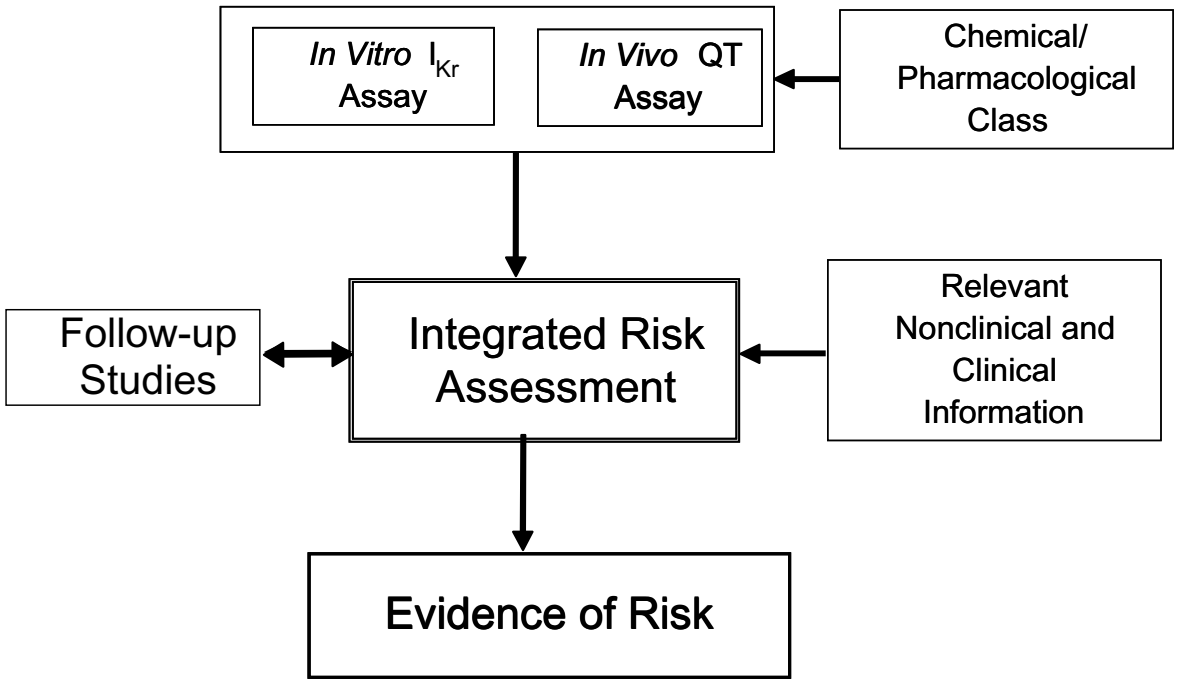
Problem Statement:

- Neither ICH S7B or E14 specifically mentions how CV safety pharmacology testing of biologics should be accomplished
  - Guidance and current trends suggest conservative expectations

Questions:

1. How to best evaluate biologics for potential to prolong QT interval?
2. How to best evaluate biologics for CV safety risks?

ICH S7B: Nonclinical Testing Strategy



In Vitro hERG Assays - MAbs

- FDA's recommendation to conduct an *in vitro* assay to assess the effects of monoclonal antibody on the I_{Kr} channel –
  - "No specific concern around [molecule target], but a nonclinical study is the path forward for not doing a QTc study."
  - "Negative results from such a study could be presented as part of a rationale for not conducting a formal clinical QTc prolongation study."

hERG assay for MAb's?

- MAb's have very low potential for interacting with the extra- or, intracellular pore domains
- Do not have ability to cross plasma membrane directly; no access to intracellular "pore"
- QTc assessment: integrate into repeat-dose toxicology studies in appropriate species

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Appraisal of state-of-the-art

Scientific review and recommendations on preclinical cardiovascular safety evaluation of biologics

Hugo M. Vargas [a,*], Alan S. Bass [b], Alexander Breidenbach [c], Hal S. Feldman [d], Gary A. Gintant [e], Alexander R. Harmer [f], Bronagh Heath [g], Peter Hoffmann [h], Armando Lagrutta [i], Derek Leishman [j], Nick McMahon [g], Scott Mittelstadt [e], Liudmila Polonchuk [c], Michael K. Pugsley [k], Joseph J. Salata [i], Jean-Pierre Valentin [f]

Amgen, Schering-Plough, Hoffman-La Roche, Wyeth, Abbott, GSK, Novartis, Merck, Lilly, Johnson & Johnson, AstraZeneca

In Vitro I_{Kr} Assays - Biologics

- Drug Trapping within the K+channel Vestibule
  - Protein biologics have limited intracellular penetration, they would not be expected to reach binding site typical of hERG blockers. Tristani-Firouzi et al. *Am J Med.* 2001

- hERG Drug-binding Site
  - Located in the central cavity of the channel and should be inaccessible to large molecules such as therapeutic protein biologics. MC Sanguinetti, M Tristani-Firouzi *Nature* 2006

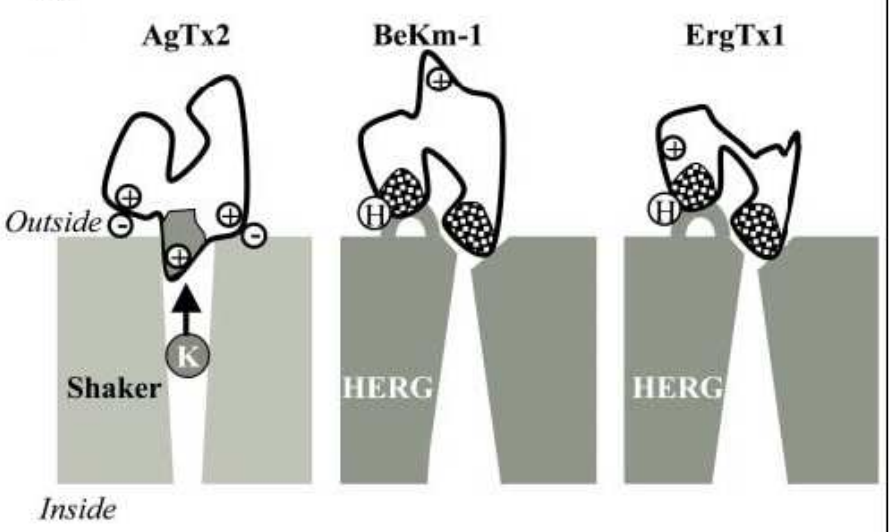
- hERG Toxin-binding Site
  - Located external to the channel, but has specific binding motifs that are unlikely to be present in most protein biologics

Monoclonal antibodies have very low potential for interactions with hERG channel

- Unable to access the “inner pore” and bind amino acid residues required to inhibit channel function
  - “Size matters”
  - Poor access to cytosol

- Unable to bind the external regions of the channel (“toxin binding site”)
  - Requirements for toxin binding are very specific, e.g., BeKm-1
  - mAb: low off-target potential

hERG Toxin Binding Site Unlikely to Bind MAb

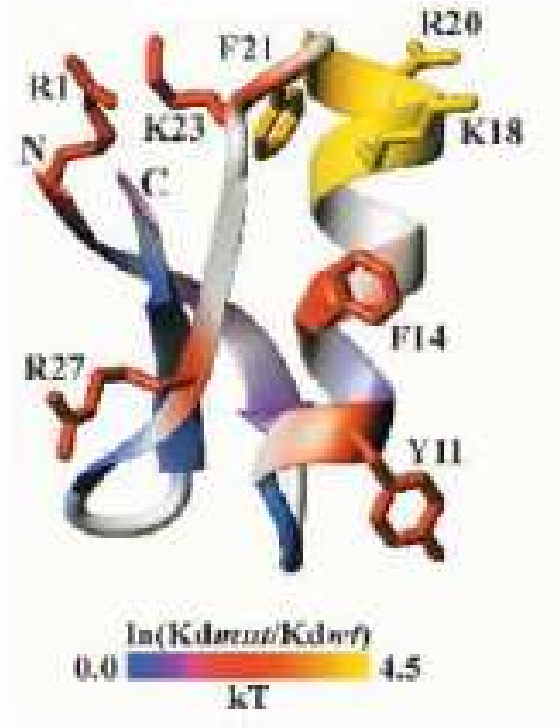


Zhang M., *Biophysical Journal,* 2003, 84:3022
Figure 11C

- Located external to the pore
- Targeted by naturally occurring peptide toxins (30-40 aa)
  - Scorpions: BeKm-1, BmTx3, CnErg1, ErgTx1
  - Sea anemones: APETx1
  - Dinoflagellates: Saxitoxin
- Interactions highly specific:
  - Highly conserved sequences on hERG (mainly S5-P segment)
  - Unique binding motifs on toxins
- Potential interaction could be evaluated *in silico*
- Such motifs unlikely exist in therapeutic MAbs, which are highly specific and selective to their biologic targets

BeKm-1: Is specific for hERG channel & has no interaction with other channels

K^+ channel	100 nM BeKm-1 (% inhibition)
Voltage-gated[a]	
Kv1.2 ($n=3$)	0
Kv1.4 ($n=3$)	0
Kv2.1 ($n=3$)	0
Kv4.3 ($n=3$)	0
Inward rectifier	
Kir1.1 ($n=3$)	0
Calcium-activated	
hSK1 ($n=3$)	0
rSK2 ($n=3$)	0
hIK ($n=3$)	0
hBK ($n=3$)	0
KCNQ	
KCNQ1/KCNE1 ($n=3$)	0
KCNQ2+3 ($n=3$)	0
KCNQ4 ($n=3$)	0
Ether-a-go-go-like	
hERG1 ($n=4$)	92 ± 2
rELK1 ($n=3$)	9 ± 3
hEAG ($n=3$)	0

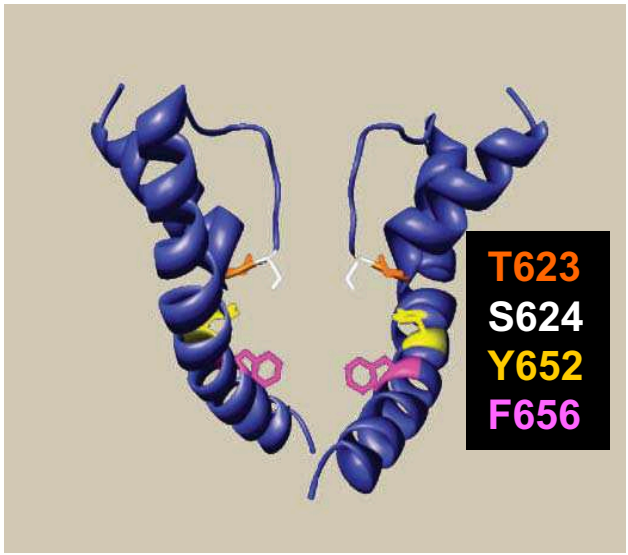


Korolkova *et al.*, J Mol Recog, 17:209, 2004

hERG Drug Binding Site Inaccessible to MAb

Common features of hERG blockers

- Small (250-600 Da)
- Access to the inner pore
- Low specificity

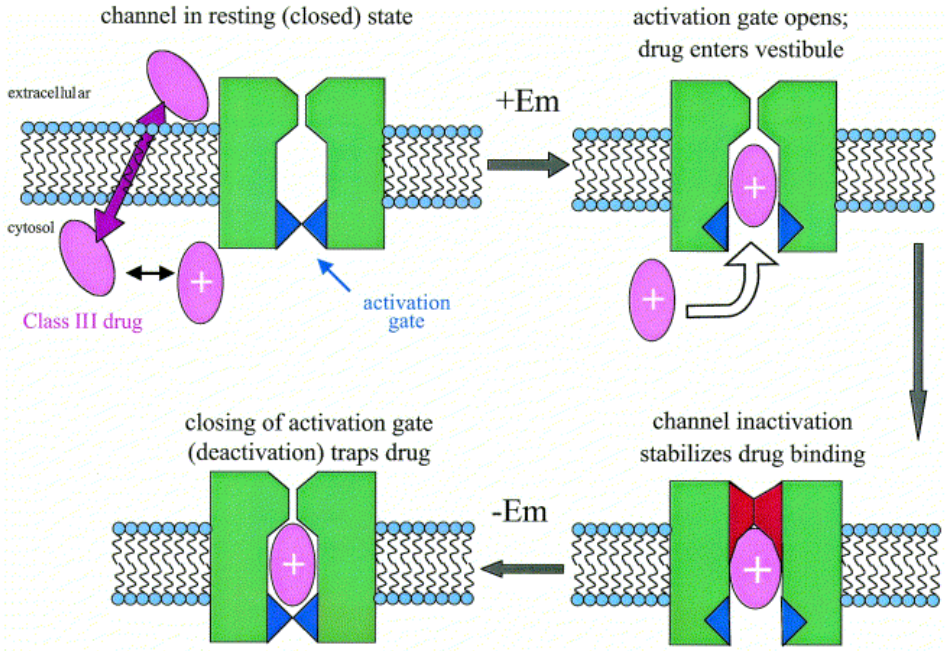


Homology model of the hERG-channel pore module based on the crystal structure of KvAP. Key residues that interact with structurally diverse drugs are shown.

- hERG drug binding site inaccessible to large molecules such as therapeutic biologics (MAb, FAb, fusion protein)
  - Size matters

Jiang Y. et al, Nature, 2003; Sanguinetti M. et al., Nature; 2006, Doyle D., et al, Science, 1998

hERG Intracellular Site Inaccessible to MAb



Tristani-Firouzi et al. *Am J Med.* 2001

- Protein biologics have limited intracellular penetration
- Not expected to reach binding site typical of hERG blockers

hERG Assay: Not Recommended for MAbs

- Mechanistically, therapeutic MAbs unlikely to affect the hERG channel
  - Unlikely to enter cells and block channel like small molecules
  - High specificity/selectivity and low off-target potential, unlikely to interact with ion channel proteins
- Test system incompatibility
  - Biologics may be formulated with excipients that are known to block the hERG channel in vitro (e.g., Tween) – confounds assay, interpretation
  - In vitro models - protein-free buffers can be expected to have negative impact on stability, activity, or test system compatibility of biologics – integrity issue
- Conclusion: no scientific rationale to perform hERG assays on MAbs
  - We do not conduct the hERG assay for MAbs
  - Similar principle applied to fusion proteins and Fab

QT/QTc Prolongation Potential of Biologics?

- Direct hERG blockade unlikely
- If present, secondary effects more likely cause
  - Oxytocin: vasodilation, hypotension, tachycardia, and transient QTc prolongation
  - Vasopressin: hypertension, bradycardia, QT prolongation, and TdP
- In vivo assessment provides a more relevant risk assessment

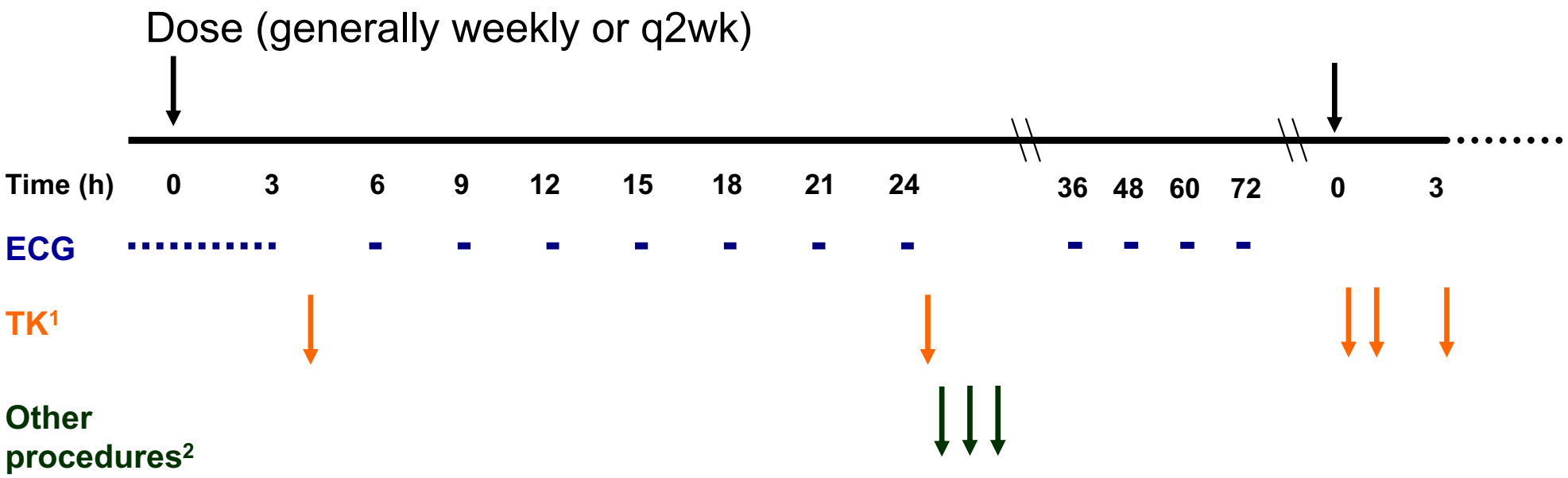
Strategy for Assessing QT Prolongation Potential of Biologics

- Preclinical: in vivo assessment of QT/QTc prolongation potential in animal studies
  - CVS assessment as part of the IND-enabling, repeat-dose toxicity study
  - Baseline, Cmax, steady state, end of dosing, and end of recovery
- Clinical: collect early-phase clinical QT/QTc data
  - Baseline, Cmax, and steady state
- Conduct integrated analysis of data
- Based on preclinical and early clinical data, formulate a strategy for overall risk assessment of QT/QTc prolongation potential in later stage clinical trials

Basic Integrated Design

- CVS as a part of repeat dose toxicology studies in NHP
  - Surgically implanted telemetry (ECG, BP, HR and body temperature) on subset of animals (*eg*, 2-3 animals/sex/group)
  - In-life procedures may interfere with telemetry reading. Can minimize by:
    - Separate animal rooms for telemetry animals
    - Spacing/staggering study procedures
    - Alternative: include satellite group (based on anticipated effects and feasibility issues)
  - External multilead ECG or JET & non-invasive blood pressure on all other animals

Spacing Out Study Procedures



[1] TK. Early TK timepoints can be obtained from non-telemeterized animals on dosing days when ECG readings are recorded, and, from telemeterized animals on subsequent dosing days when ECG readings are not recorded.
[2] Ophthalmology, physical examination, pulse oximetry, and neurological evaluation

Respiratory System: Basic Testing Approaches

- Respiratory System
  - Insufficient to employ clinical observation as only means for assessing respiratory function
  - Integrate auscultation, respiratory rate, and pulse oximetry or blood gas measurements into repeat-dose NHP (or other suitable non-rodent) study
  - Can also be integrated into separate CV safety study in conscious, instrumented non-rodents, if deemed appropriate

Central and Peripheral Nervous System: Basic Testing Approaches

- Central Nervous System:
  - General observations in repeat-dose NHP (or other suitable non-rodent) study
    - Behavior, coordination, motor activity, reflexes?
    - Limited evaluation because of need to anesthetize animals for most manipulations
  - If CNS penetration, and cross-reactive in rodents, can incorporate FOB or Irwin-like testing into study of appropriate duration

Basic Testing Approaches: Second Tier Safety Pharmacology

- "Second-Tier" Organ Systems
  - Gastrointestinal
  - Renal
  - Immune (activation /suppression)

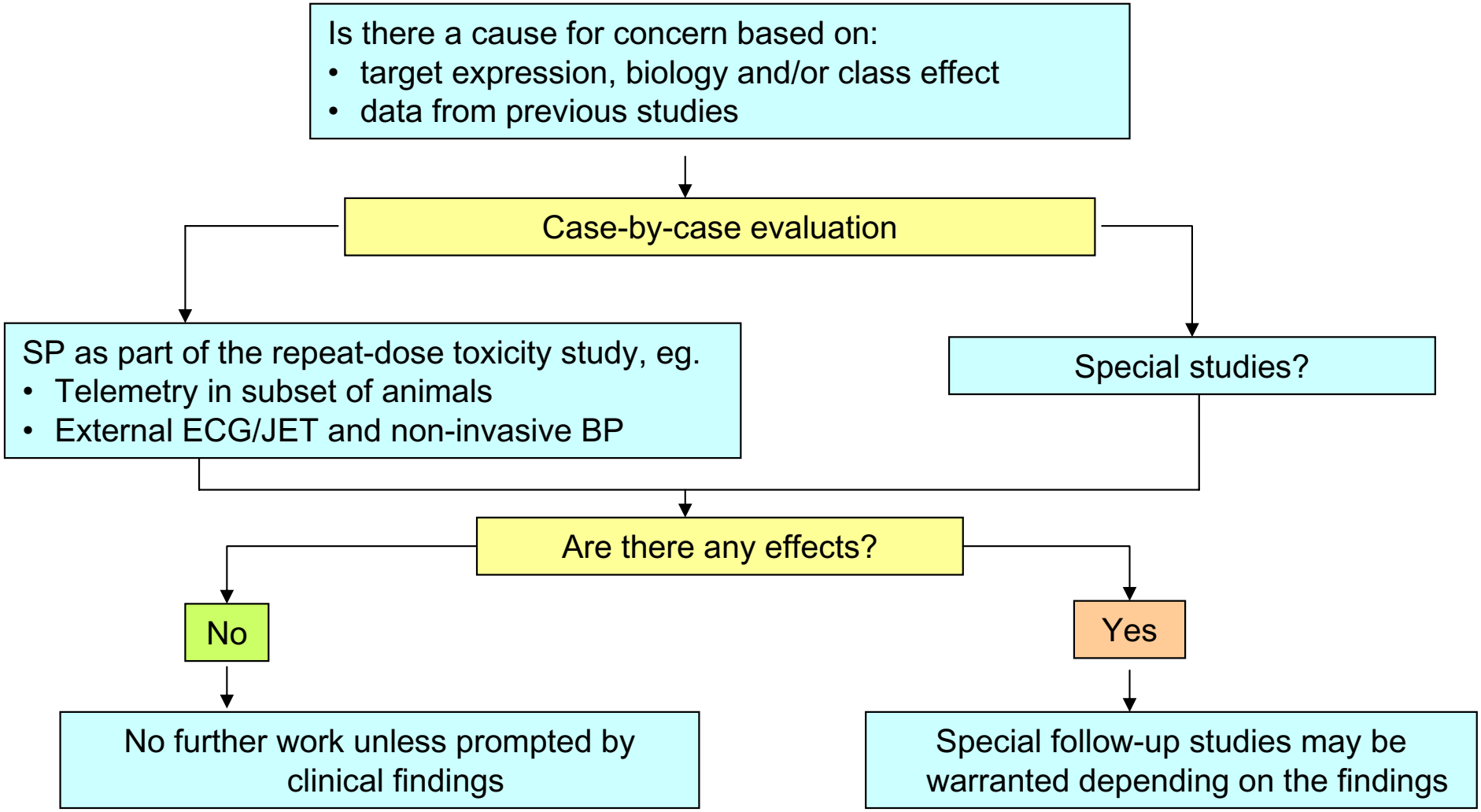
  - Concern typically driven by biology of molecule/target, clinical trial design, and/or patient population
  - Consider integrating appropriate endpoints and measurements into repeat-dose NHP (or other suitable non-rodent) study
  - Alternatively, evaluate in a separate study specifically designed to assess impact on that system/function

Safety Pharm Strategy for Biologics

- Principles:
  - Consistent with ICH S6 and S7A guidelines
  - Risk-based and data-driven
    - Target liabilities (target expression, biological activities and/or pharmacological class, etc)
    - Relevance of models and data (relevance of MOT to human, underlying diseases, co-meds, etc)
  - Consistent with ‘3R’ (replace, reduce, refine) approaches

- Approaches
  - Incorporate, as feasible, into repeat-dose toxicity studies
  - Specialized studies/endpoints may be appropriate based on target liabilities
  - Follow-up studies as needed based on emergent findings

Recommended Preclinical Testing Strategy



Conclusions

- Similar sensitivities about the potential for unanticipated or undesirable pharmacologic effects with both small molecules and biologics

- The predictive assays available to help evaluate these risks are essentially comparable, but the strategies and exact methods employed can vary considerably, especially for biologics.

Conclusions

- Biologicals are unique therapeutics
  - High target specificity; low off-target toxicity
  - Advantages over SM therapeutics: attractive
- Differences between LM and SM are clear
  - Preclinical development & Regulatory
  - Different development pathways: allowed
- Appropriate to integrate SP endpoints into toxicology studies
  - No scientific basis for conducting hERG assay
  - ECG/CNS/Respiratory in repeat-dose toxicology

Conclusions

- Safety pharmacology program design for biologics should be driven by:
  - Nature, biology, physicochemical characteristics, and species specificity of the molecule
  - Biology and expression of target
  - Availability of relevant model
  - Assay conditions and formulation components
  - Scientific rationale
  - Feasibility
  - Ability to interpret the data generated and to answer the questions being posed
- Be flexible, not dogmatic in approach
- Do what's best for the patient

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