NTP
National Toxicology Program

A Novel Framework for Human-relevant and Failure Mode-based Assessment of Cardiovascular Safety in Nonclinical Drug Development

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29 Mar 2019

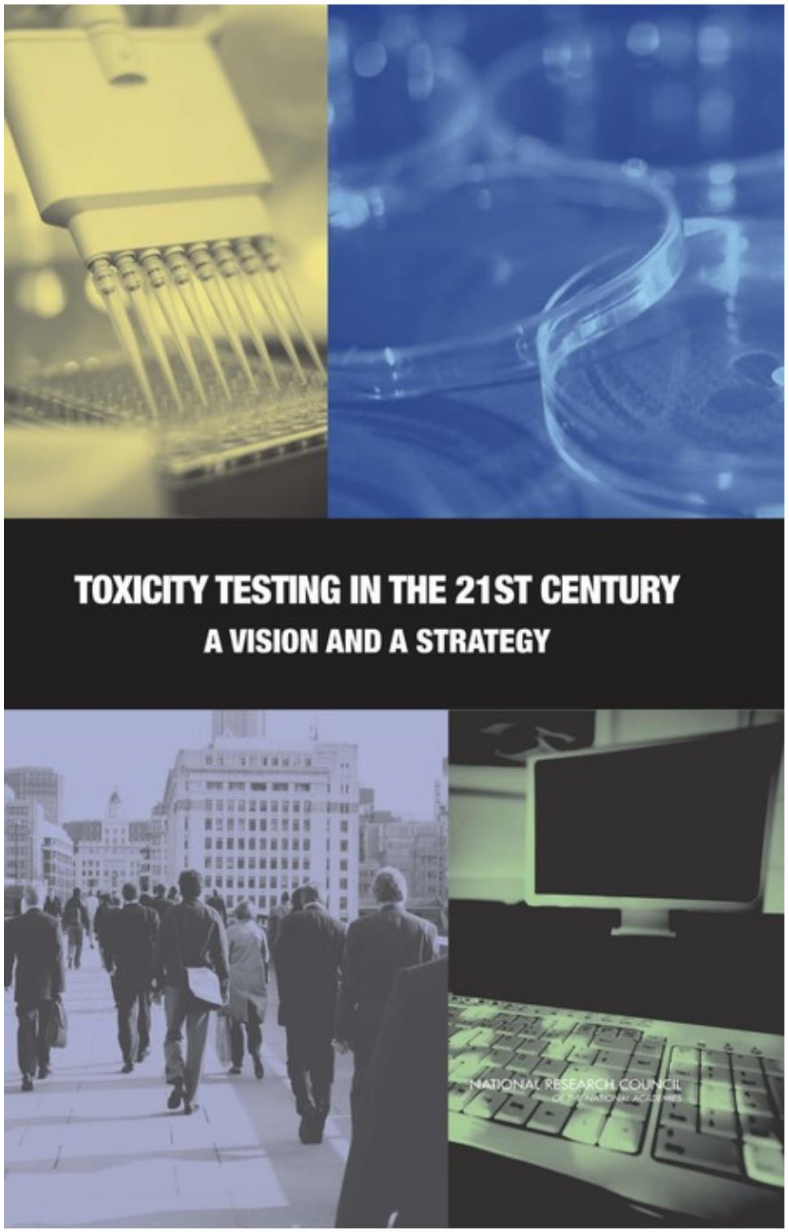
Outline

- Rationale for a novel approach
- Aims and value proposition
- Enablers
- Strategic approach
- Challenges

Rationale

- Drug development attrition is a significant challenge
- Safety-related attrition a significant contributor
- Cardiovascular liabilities identified in animal studies late in development are prominent
  - source of attrition prior to clinical testing
- Cardiovascular liabilities identified in patients are worse
  - most problematic liabilities are those associated with imbalances in MACE
- Animal liabilities may or may not be human liabilities

Related Needs



NRC Committee on Toxicity Testing and Assessment of Environmental Agents

"Toxicity testing is under increasing pressure to meet several competing demands:

- Test large numbers of existing chemicals, many of which lack basic toxicity data.
- Test the large number of new chemicals and novel materials, such as nanomaterials, introduced into commerce each year.
- Evaluate potential adverse effects with respect to all critical end points and life stages.
- Minimize animal use.
- Reduce the cost and time required for chemical safety evaluation.
- Acquire detailed mechanistic and tissue-dosimetry data needed to assess human risk quantitatively and to aid in regulatory decision-making.

National Academies Press, 2007

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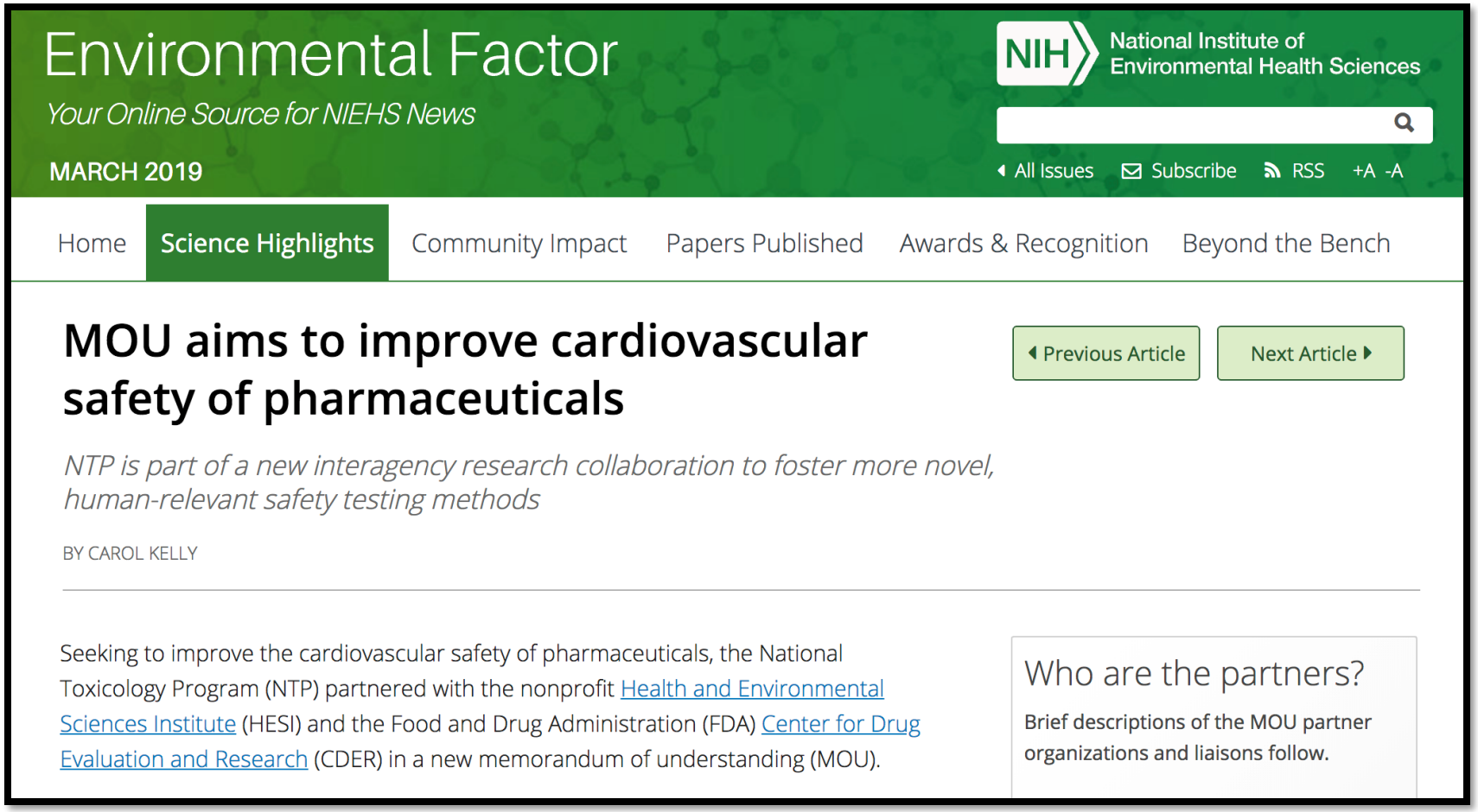
National Toxicology Program

Mechanistic, Human-relevant Cardiovascular Safety Assessment: A HESI Cardiac Safety Technical Committee Initiative

2015?
April, 2018

Innovation Partners



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MOU aims to improve cardiovascular safety of pharmaceuticals

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NTP is part of a new interagency research collaboration to foster more novel, human-relevant safety testing methods

BY CAROL KELLY

Seeking to improve the cardiovascular safety of pharmaceuticals, the National Toxicology Program (NTP) partnered with the nonprofit Health and Environmental Sciences Institute (HESI) and the Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) in a new memorandum of understanding (MOU).

Who are the partners?

Brief descriptions of the MOU partner organizations and liaisons follow.

Mission Statement

Contemporary pharmaceutical cardiovascular safety assessment would benefit from an approach that is more efficient in cost and time, mechanistically informative and human relevant. Such an approach would enable earlier recognition of development-limiting liabilities, fewer false positives leading to premature development termination, more relevant biomarkers and decreased late-stage attrition. The HESI Cardiac Safety Technical Committee will work across its working groups and with other stakeholders to design, test and implement such an approach.

Mission Statement

Aim

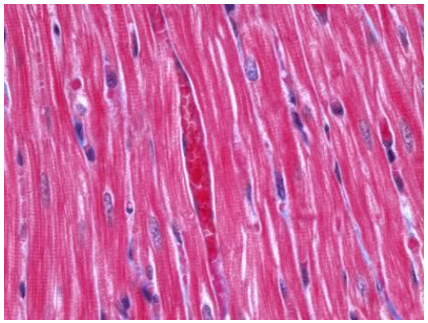
Value proposition

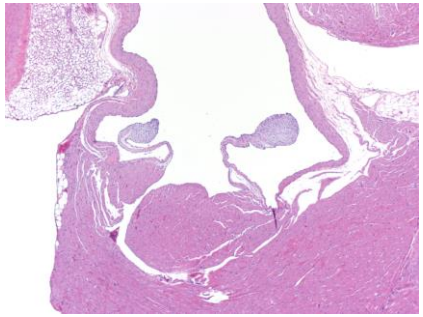
Contemporary pharmaceutical cardiovascular safety assessment would benefit from an approach that is more efficient in cost and time, mechanistically informative and human relevant. Such an approach would enable earlier recognition of development-limiting liabilities, fewer false positives leading to premature development termination, more relevant biomarkers and decreased late-stage attrition. The HESI Cardiac Safety Technical Committee will work across its working groups and with other stakeholders to design, test and implement such an approach.

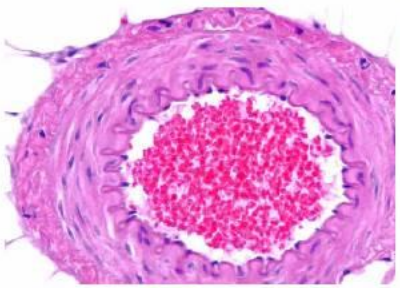
Key Assumptions

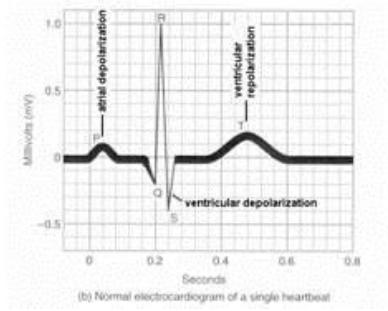
- There are a finite number of primary responses to CV toxicity- i.e. failure modes
- Behind those failure modes, there are a finite number of key cellular and or molecular ‘mechanistic’ events (modes of action) that initiate and drive their pathogenesis which are ‘screenable’
- The likelihood of a xenobiotic inducing a failure mode is a product of it’s potency for functionally perturbing a cellular event and the likely *in vivo* exposure in dose and time
  - our confidence in a phenotypic outcome for a mechanistic activity relates to our experience with it- i.e. some activity at a mechanistic level can be directly related to a phenotypic outcome (e.g. 5HT2b agonism)
  - other activities will require phenotypic confirmatory testing (i.e. Tier 2) in more complex biological systems to build confidence in the phenotypic outcome
- A relevant mechanistic testing strategy should enable clinical risk assessment, progression decisions and the development of clinical monitoring strategies

Feasibility: We know what the CV system looks like and how it works!

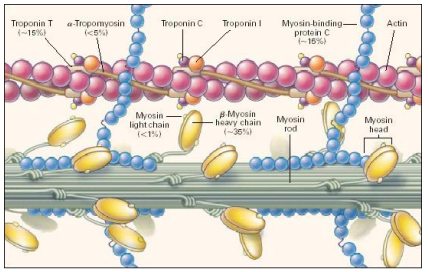


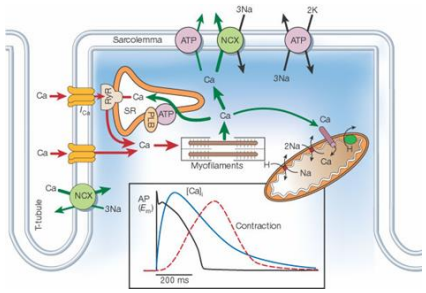


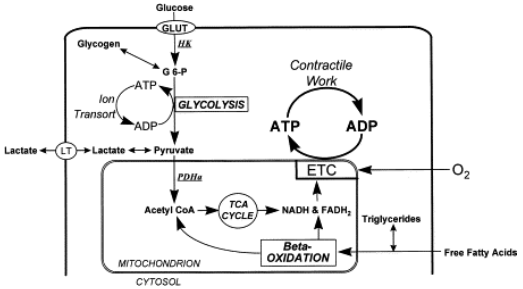


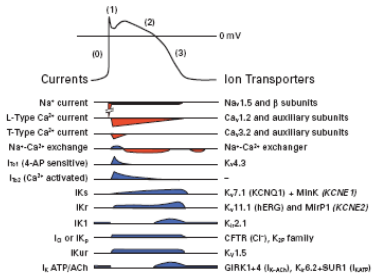


It's plumbing, electromechanics and energetics!

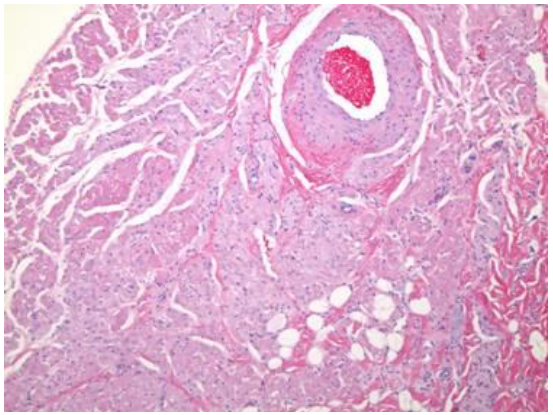








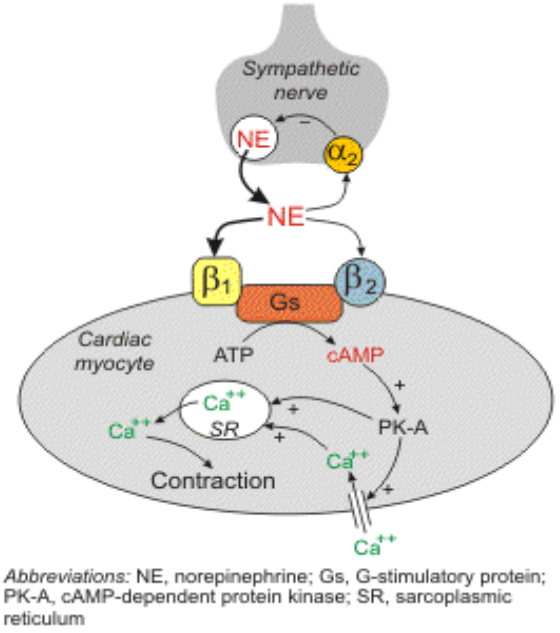
Feasibility: We understand many control systems!



Frank-Starling Law

Natriuretic peptides

- β-adrenergic agonist
  - non-selective for β_1, β_2
  - β_1 = ↑ cardiac inotropy, chronotropy
  - β_2 = vasodilation

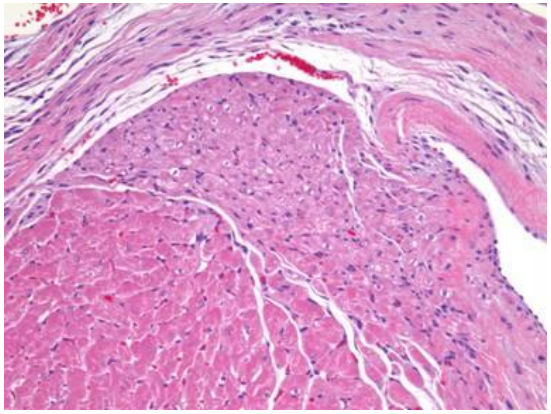


Abbreviations: NE, norepinephrine; Gs, G-stimulatory protein; PK-A, cAMP-dependent protein kinase; SR, sarcoplasmic reticulum

- Heart rate (chronotropy) determined by rate of spontaneous SA nodal discharge
- Spontaneous SA nodal discharge determined by balance of autonomic control

Sympathetic-	norepinephrine	↑ discharge
Parasympathetic-	acetylcholine	↓ discharge

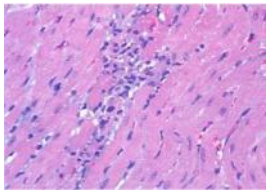
Renin-angiotensin system



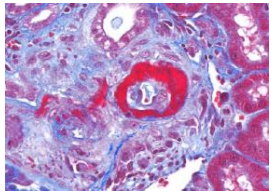
NO, Endothelin

Feasibility: We know what cardiovascular toxicity looks like!

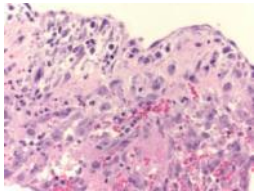
Structural injuries



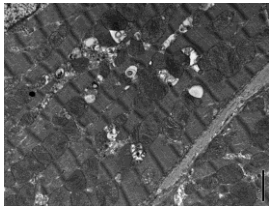
cardiomyocyte injury



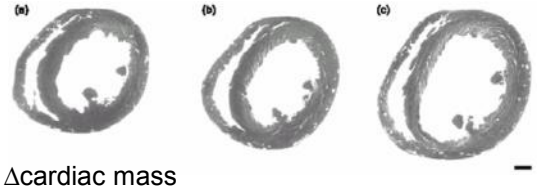
vascular injury



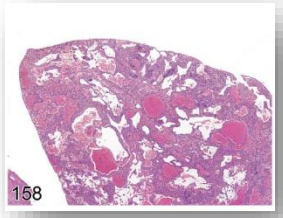
valvulopathy



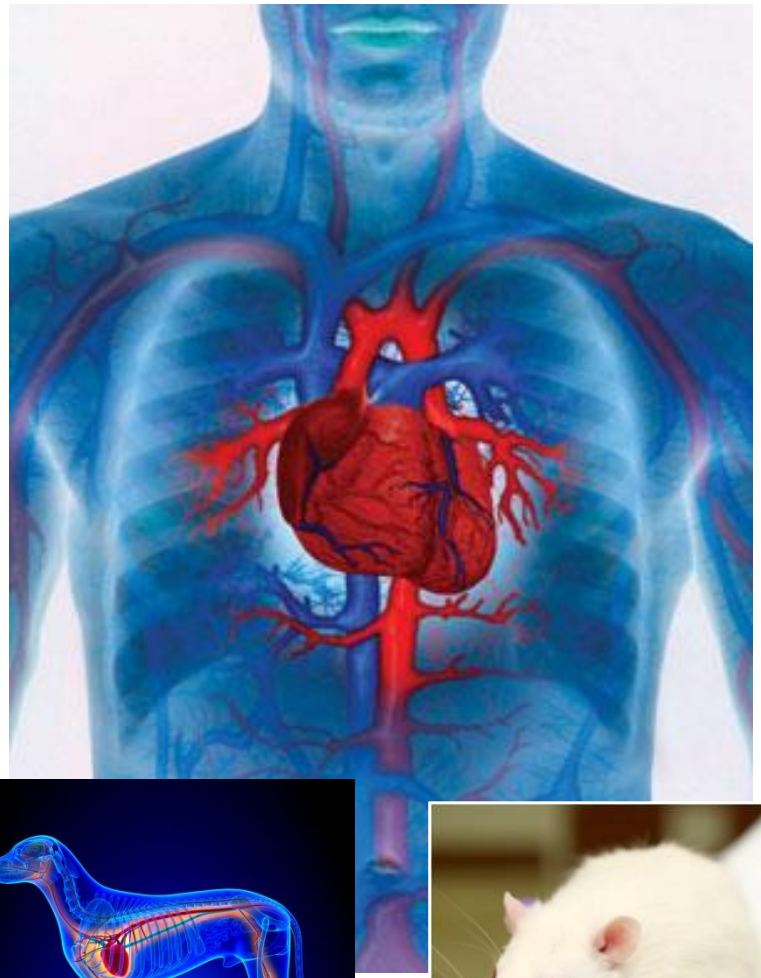
organellar injury

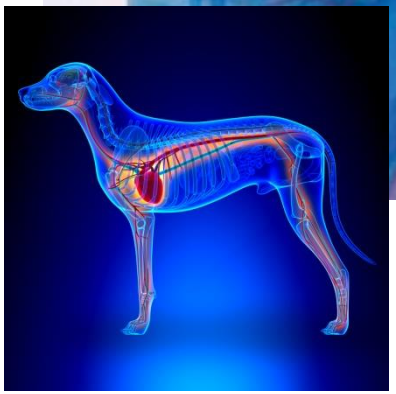


Δcardiac mass

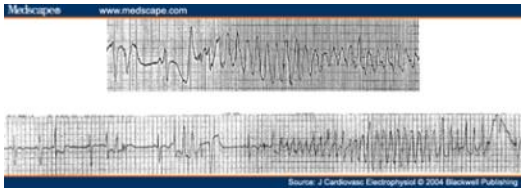


Neoplasia





Functional changes



Arrhythmia

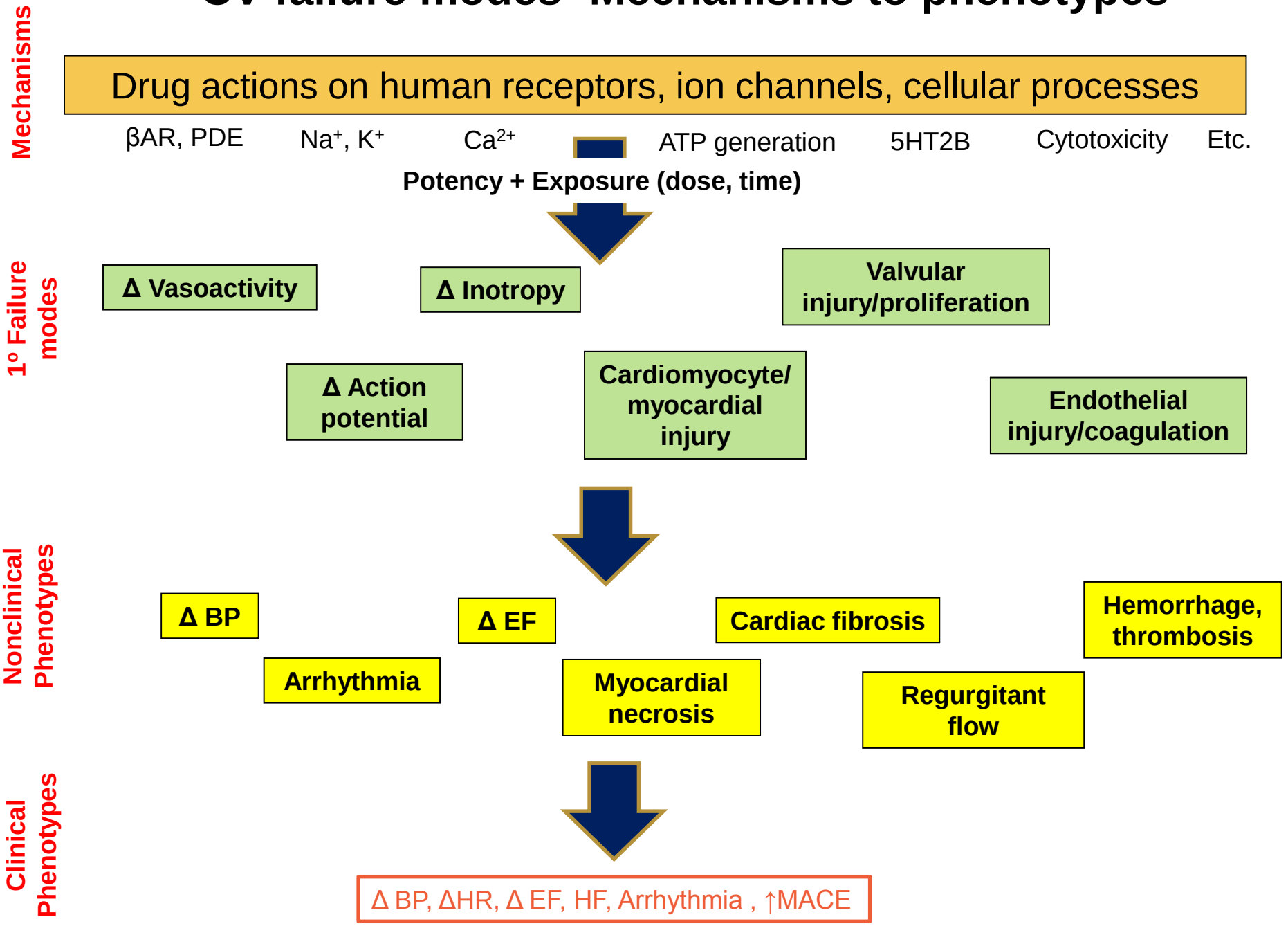
Δ BP Δ HR

Δ contractility

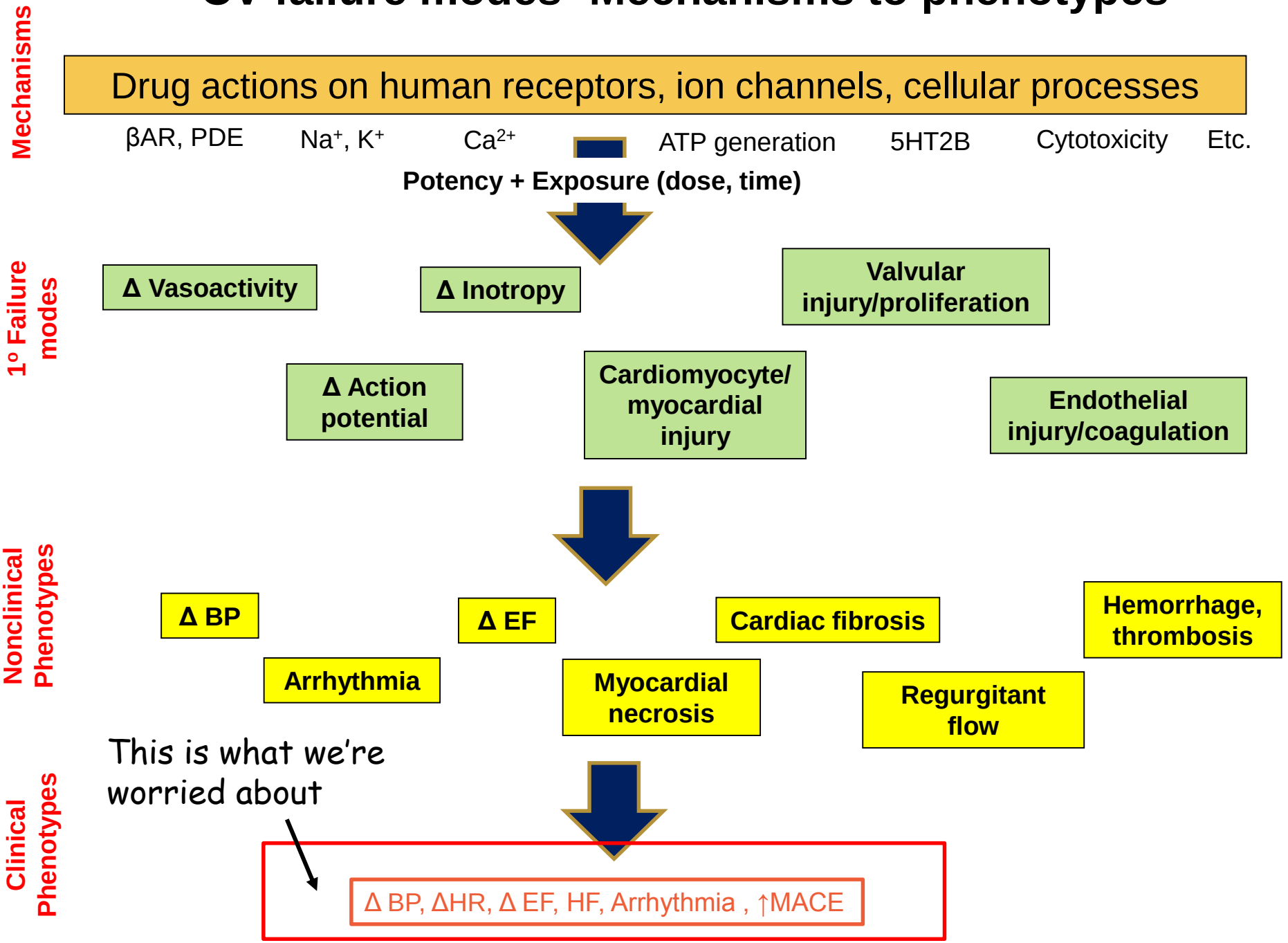
Changes in disease

Ischemic events
Coronary artery dz
Heart failure
Cerebrovascular events
Hypertension
Metabolic disease

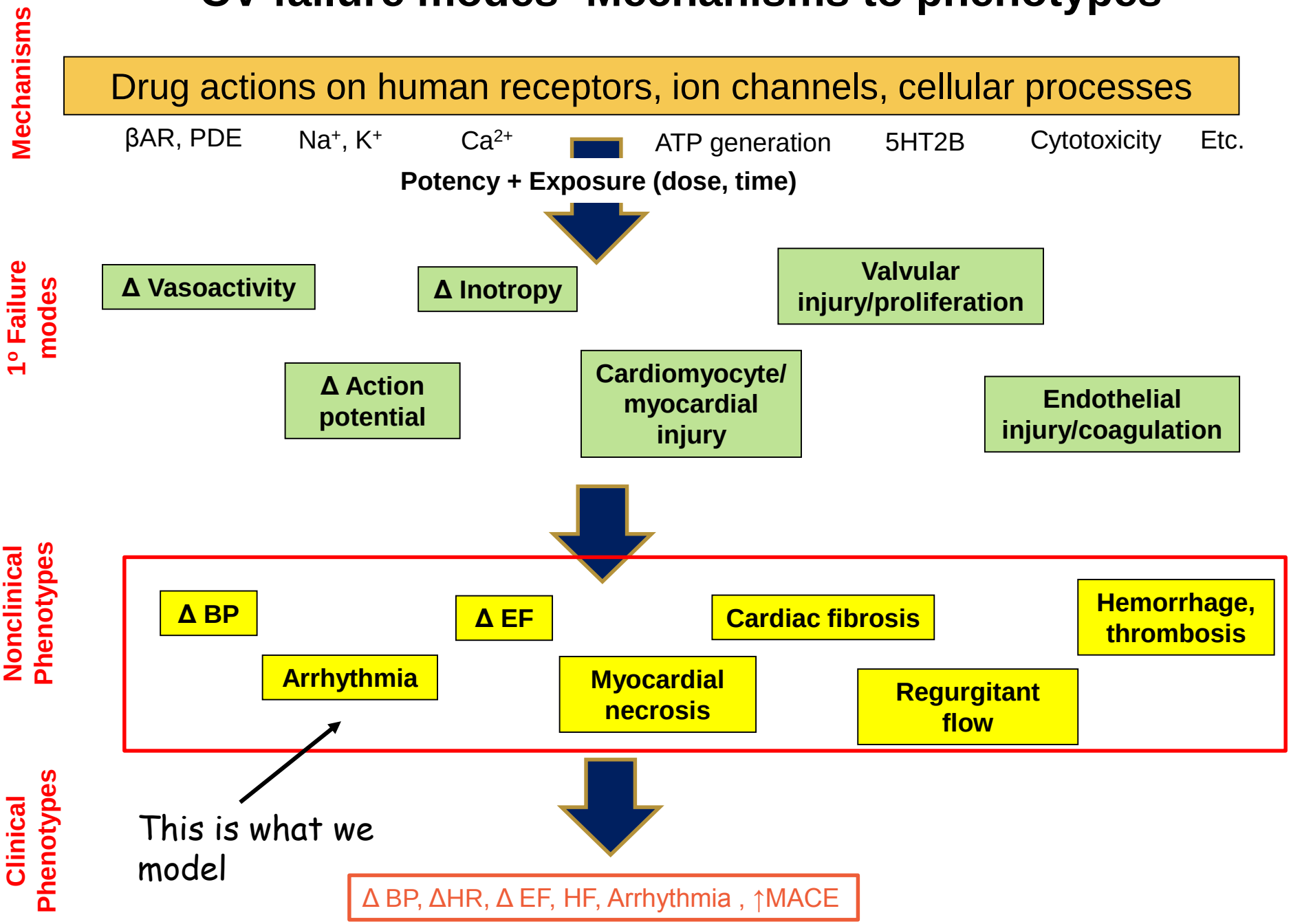
CV failure modes- Mechanisms to phenotypes



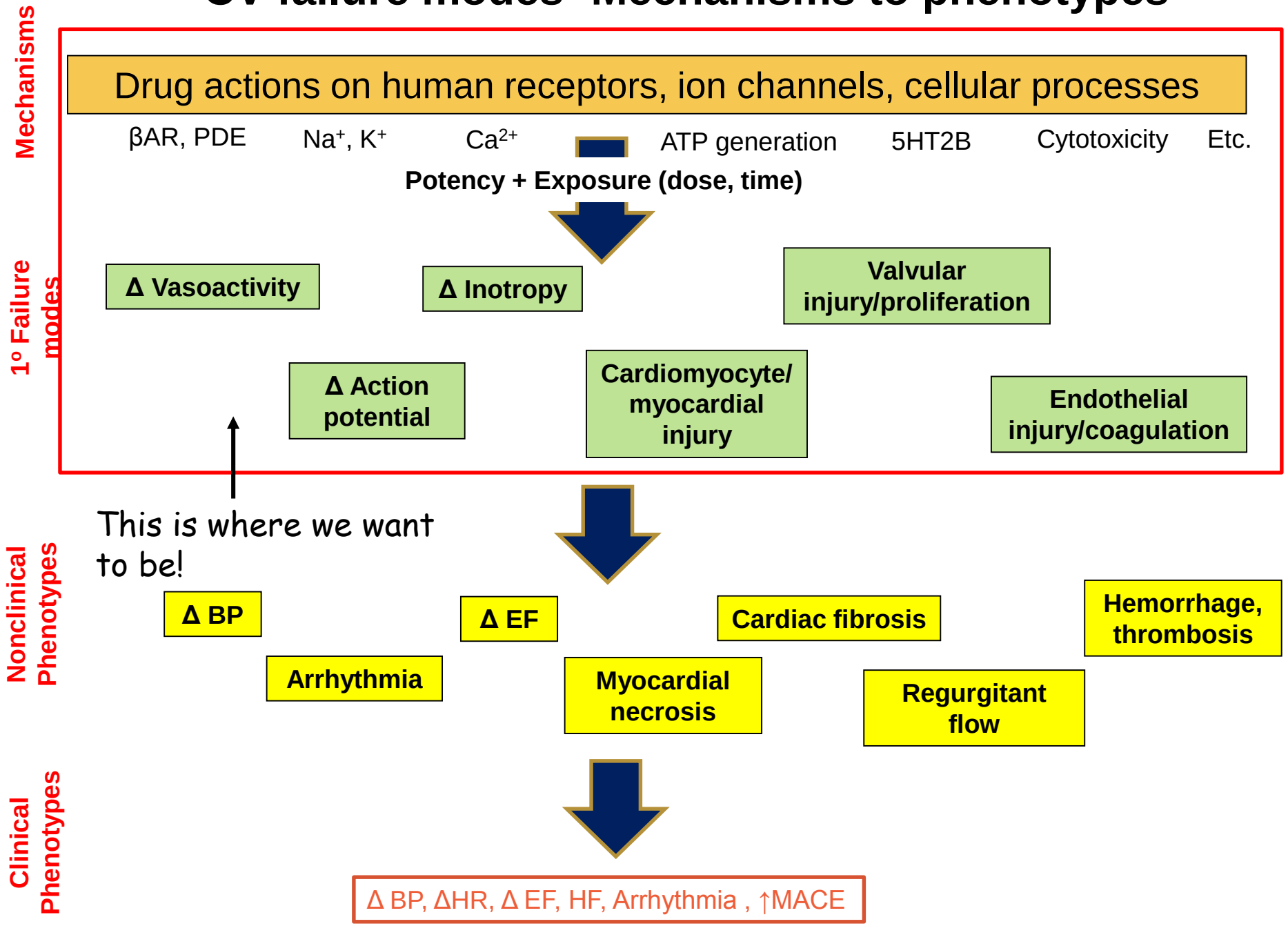
CV failure modes- Mechanisms to phenotypes



CV failure modes- Mechanisms to phenotypes

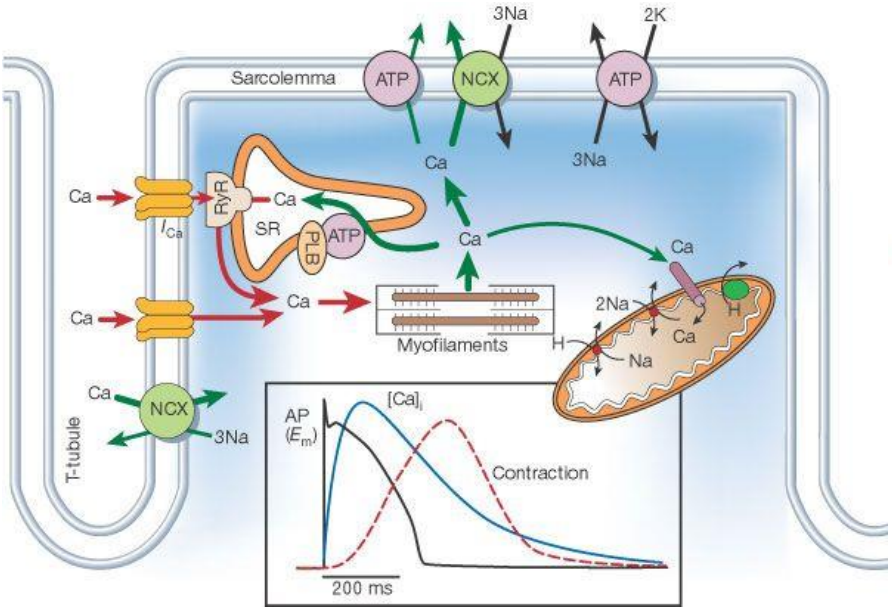


CV failure modes- Mechanisms to phenotypes

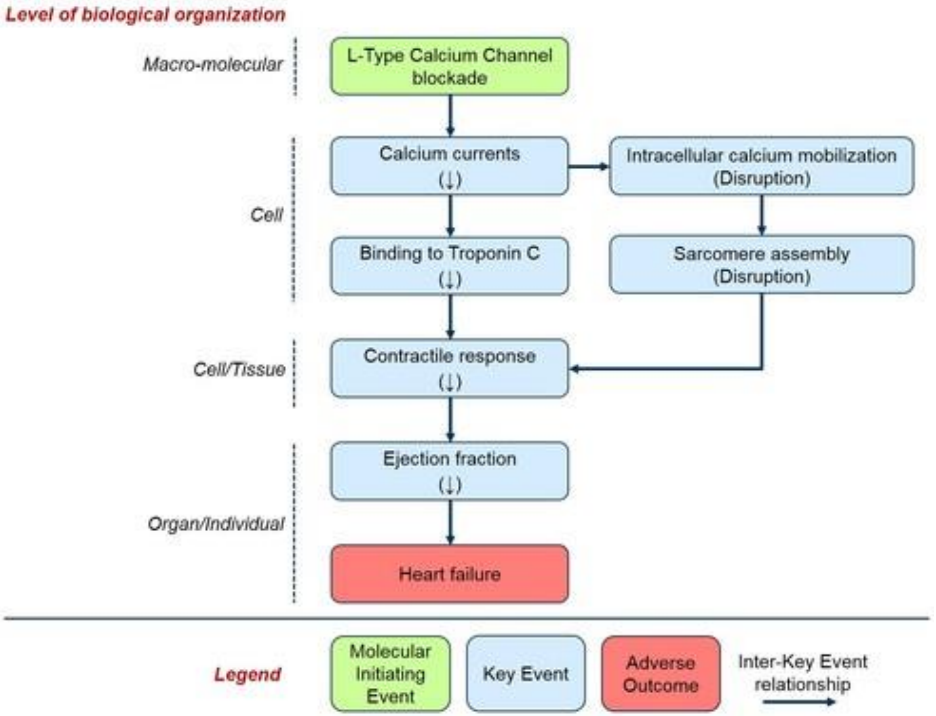


Do AOPs have a role in this?

Excitation-contraction coupling



Calcium channel blockade and heart failure



Mechanistic screening isn't new!

A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Are there other targets we should be adding to this primary screen?

Table 1 | **Recommended targets to provide an early assessment of the potential hazard of a compound or chemical series**

Targets (gene)	Hit rate*		Main organ class or system	Effects		Refs§
	Binding	Functional or enzymatic		Agonism or activation	Antagonism or inhibition	
G protein-coupled receptors						
Adenosine receptor A_{2A} (*ADORA2A*)	High	Low (agonist)	CVS, CNS	Coronary vasodilation; ↓ in BP and reflex; ↑ in HR; ↓ in platelet aggregation and leukocyte activation; ↓ in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; ↑ in BP; nervousness (tremors, agitation); arousal; insomnia	57
α_{1A}-adrenergic receptor (*ADRA1A*)	High	Low (agonist); high (antagonist)	CVS, GI, CNS	Smooth muscle contraction; ↑ in BP; cardiac positive ionotropy; potential for arrhythmia; mydriasis; ↓ in insulin release	↓ in smooth muscle tone; orthostatic hypotension and ↑ in HR; dizziness; impact on various aspects of sexual function	58
α_{2A}-adrenergic receptor (*ADRA2A*)	High	Low (agonist); medium (antagonist)	CVS, CNS	↓ in noradrenaline release and sympathetic neurotransmission; ↓ in BP; ↓ in HR; mydriasis; sedation	↑ in GI motility; ↑ in insulin secretion	59
β_1-adrenergic receptor (*ADRB1*)	Medium	NA	CVS, GI	↑ in HR; ↑ in cardiac contractility; electrolyte disturbances; ↑ in renin release; relaxation of colon and oesophagus; lipolysis	↓ in BP; ↓ in HR; ↓ in CO	60
β_2-adrenergic receptor (*ADRB2*)‡	High	Medium (agonist); medium (antagonist)	Pulmonary, CVS	↑ in HR; bronchodilation; peripheral vasodilation and skeletal muscle tremor; ↑ in glycogenolysis and glucagon release	↓ in BP	61
Cannabinoid receptor CB_1 (*CNR1*)	Medium/ high	Medium (antagonist)	CNS	Euphoria and dysphoria; anxiety; memory impairment and poor concentration; analgesia; hypothermia	↑ in weight loss; emesis; depression	62
Cannabinoid receptor CB_2 (*CNR2*)	Medium	Medium (agonist)	Immune	Insufficient information	↑ in inflammation; ↓ in bone mass	63
Cholecystokinin A receptor (*CCKAR*)	Low/ medium	NA	GI	↓ in food intake; gallbladder contraction; pancreatic enzyme secretion; ↑ in GI motility; activation of dopamine-mediated behaviour	↑ in development of gallstones	64
Dopamine receptor D_1 (*DRD1*)‡	Medium/ high	Medium (antagonist)	CVS, CNS	Vascular relaxation; ↓ in BP; headaches; dizziness; nausea; natriuresis; abuse potential	Dyskinesia; parkinsonian symptoms (tremors); anti-emetic effects; depression; anxiety; suicidal intent	65
Dopamine receptor D_2 (*DRD2*)‡	Medium/ high	Medium/high (agonist); medium (antagonist)	CVS, CNS, endocrine	↓ in HR; syncope; hallucinations; confusion; drowsiness; ↑ in sodium excretion; emesis; ↓ in pituitary hormone secretions	Orthostatic hypotension; drowsiness; ↑ in GI motility	66
Endothelin receptor A (*EDNRA*)	Low	NA	CVS, development	↑ in BP; aldosterone secretion; osteoblast proliferation	Teratogenicity	67

Table 1 (cont.) | **Recommended targets to provide an early assessment of the potential hazard of a compound or chemical series**

Targets (gene)	Hit rate*		Main organ class or system	Effects		Refs§
	Binding	Functional or enzymatic		Agonism or activation	Antagonism or inhibition	
G protein-coupled receptors (cont.)						
Muscarinic acetylcholine receptor M_1 (*CHRM1*)	High	Low (agonist); high (antagonist)	CNS, GI, CVS	Proconvulsant; ↑ in gastric acid secretion; hypertension; tachycardia; hyperthermia	↓ in cognitive function; ↓ in gastric acid secretion; blurred vision	73
Muscarinic acetylcholine receptor M_2 (*CHRM2*)‡	High	Low (agonist); medium (antagonist)	CVS	↓ in HR; reflex; ↑ in BP; negative chronotropy and inotropy; ↓ in cardiac conduction (PR interval); ↓ in cardiac action potential duration	Tachycardia; bronchoconstriction; tremors	74
Muscarinic acetylcholine receptor M_3 (*CHRM3*)	High	NA	GI, pulmonary	Bronchoconstriction; ↑ in salivation; GI and urinary smooth muscle constriction	Constipation; blurred vision; pupil dilation; dry mouth	75
5-HT_{1A} (*HTR1A*)	Medium/ high	Low (agonist); medium (antagonist)	CNS, endocrine	↓ in body temperature; reduced REM sleep; ↑ in ACTH; cortisol and growth hormone secretion	Potentially anxiogenic	76
5-HT_{1B} (*HTR1B*)	High	High (agonist); medium (antagonist)	CVS, CNS	Cerebral and coronary artery vasoconstriction; ↑ in BP	↑ in aggression	77
5-HT_{2A} (*HTR2A*)‡	Very high	Low/medium (agonist); medium/high (antagonist)	CVS, CNS	Smooth muscle contraction; platelet aggregation; potential memory impairments; hallucinations; schizophrenia; serotonin syndrome	Insufficient information	78
5-HT_{2B} (*HTR2B*)	High/ very high	Low (agonist); high (antagonist)	CVS, pulmonary, development	Potential cardiac valvulopathy; pulmonary hypertension	Possible cardiac effects, especially during embryonic development	79
Vasopressin V_{1A} receptor (*AVPR1A*)	Medium	High	Renal, CVS	Water retention in body; ↑ in BP; ↓ in HR; myocardial fibrosis; cardiac hypertrophy; hyponatraemia	Insufficient information	80
Ion channels						
Acetylcholine receptor subunit α1 or α4 (*CHRNA1* or *CHRNA4*)‡	Medium/ high	Low (opener); very high (blocker)	CNS, CVS, GI, pulmonary	Paralysis; analgesia; ↑ in HR; palpitations; nausea; abuse potential	Muscle relaxation; constipation; apnoea; ↓ in BP; ↓ in HR	81
Voltage-gated calcium channel subunit α Cav1.2 (*CACNA1C*)‡	NA	Medium/high (blocker)	CVS	Insufficient information	Vascular relaxation; ↓ in BP; ↓ in PR interval; possible shortening of QT interval of ECG	82

Designing and executing the framework

Mobilize experts in CV toxicology and safety assessment

Map phenotypic outcomes of CV toxicity (i.e. failure modes) linked to cellular targets and known mechanistic pathogeneses

Define a portfolio of potential testing platforms- e.g. binding assays vs. cellular function assays vs. 3D tissues

Crowd source the development of the needed assays

Validate the assays and **qualify** the paradigm

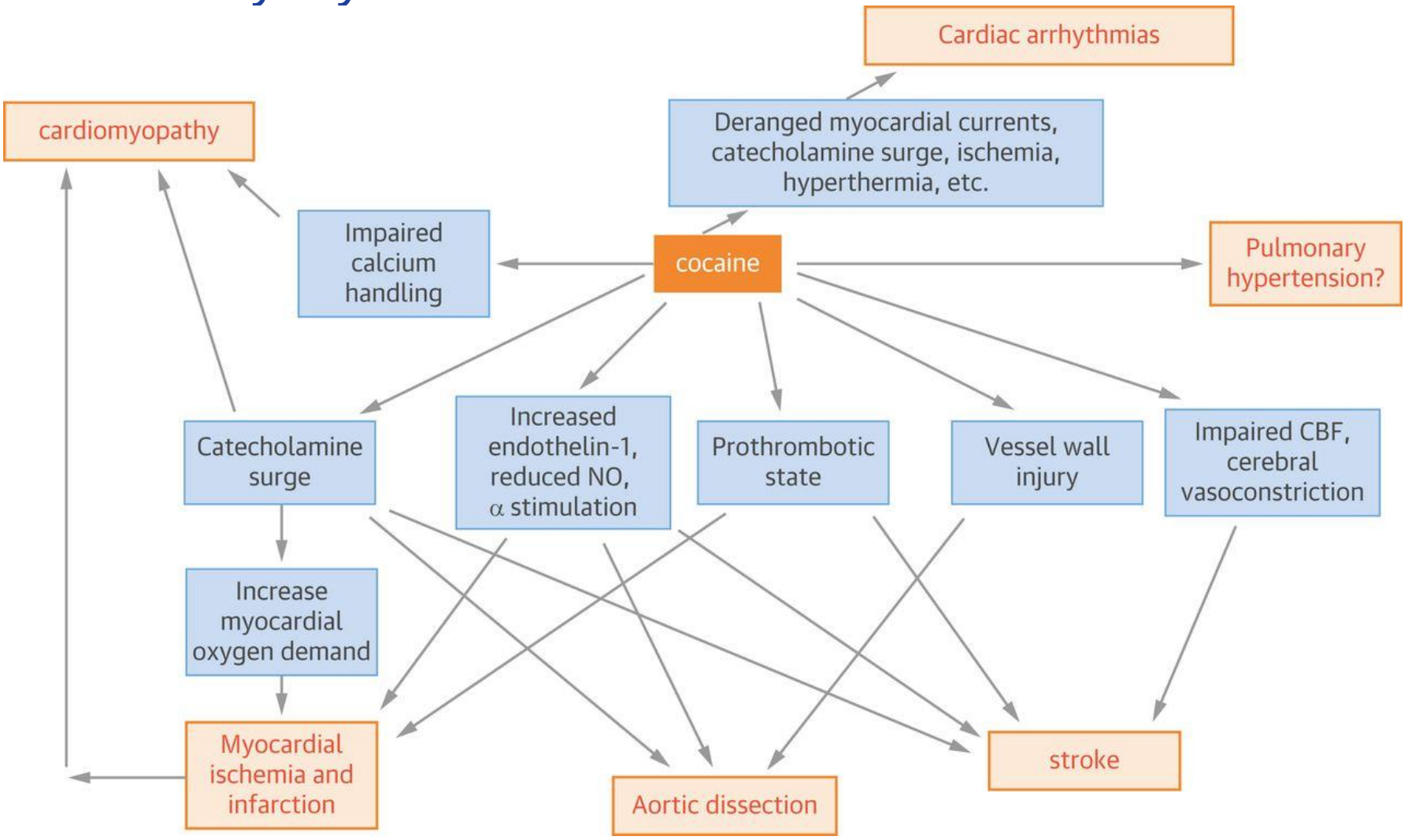
Socialize and **launch**

Salient features of the framework

- Knowledge-based
  - aligned to what we know about the mechanisms, pathogeneses and phenotypes of CV toxicity
- Human-relevant
  - systems that reflect human biology at the subcellular, cellular or tissue level
  - testing at in vivo concentrations/exposures
- Mechanisms
  - goes beyond phenotypic outcomes and probes underlying cellular mechanisms
- Ability to be applied earlier in development than traditional animal studies (e.g. at molecular design rather than candidate profiling)

The Hard Stuff- Complex pathogeneses

How many of these events are likely to be identified in a cardiomyocyte model?



The stuff that scares us: How many 'mechanisms' do we truly understand?

Doxorubicin cardiotoxicity- two (of many?) mechanisms:

- Oxidative stress
- Top2B inhibition

But, that's okay because we know a lot about 'modes'!

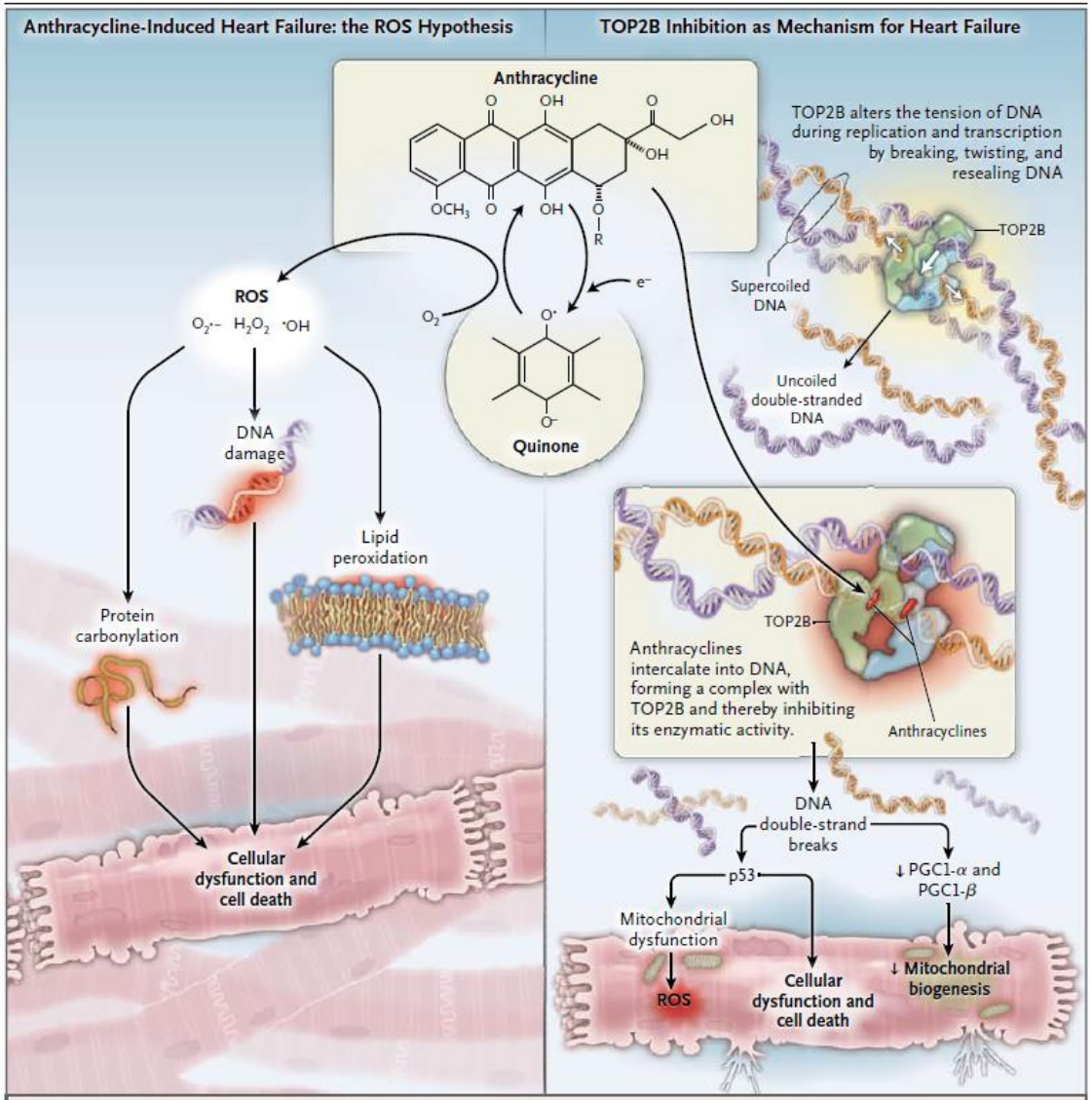
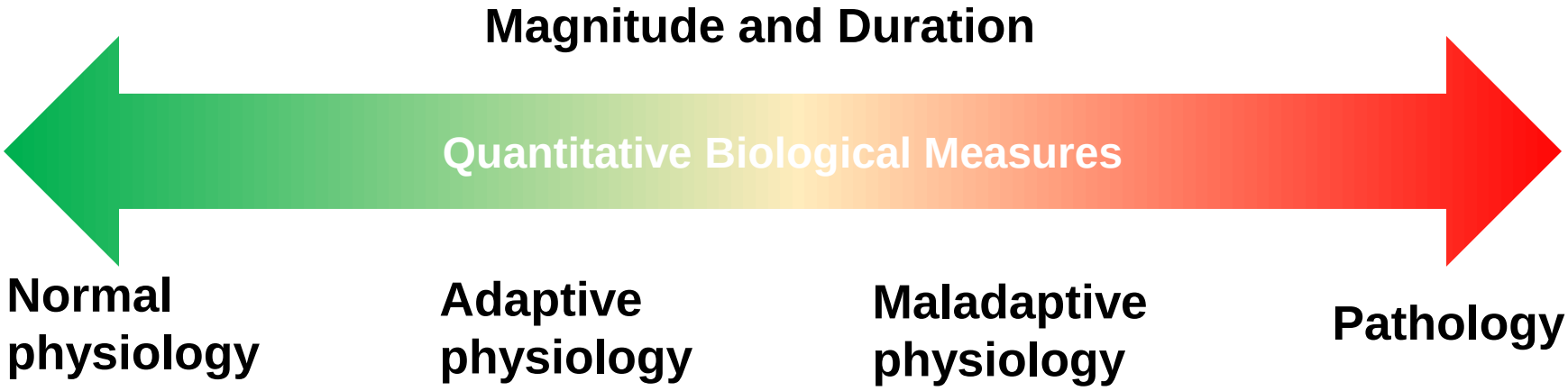


Figure 1. Mechanisms of Anthracycline-Induced Injury to Cardiac Cells.

The classic model of anthracycline cardiotoxicity involves the generation of reactive oxygen species (ROS) by the quinone moiety common to all anthracyclines. An alternative model, supported by a recent study by Zhang et al.,[2] posits that toxicity is caused by the disabling of the function of topoisomerase II beta (TOP2B) by the anthracyclines. Without functional TOP2B, double-stranded DNA breaks accrue, leading to events such as the activation of p53 tumor-suppressor protein, mitochondrial dysfunction, and the generation of ROS that result in cardiac cell death. PGC1-α and PGC1-β denote peroxisome-proliferator–activated receptor α coactivator 1α and 1β.

N ENGL J MED 368;12 NEJM.ORG MARCH 21, 2013

Key Challenges- Pathobiology is a continuum



- Transition from normal to abnormal is generally not binomial.
- Thresholds of biological perturbation that represent ‘toxicity’ are difficult to define and not generally well understood mechanistically.
- Contextualizing those perturbations in a myriad of possible individual susceptibilities is even more difficult.

Key Challenges- Validation-Qualification Continuum

Building confidence

Analytical validation

Translational qualification

Key Enablers

- replicate biology
- demonstrate pharmacology and toxicology
- test for analytical reproducibility
- comparative studies
- evolution of use
- learn to make decisions
- clinical outcomes
- tincture of time/experience

Questions?