Use of cardiomyocyte models for the detection of cardiac contractility: the opportunity and future potential

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FDA Myocytes Workshop

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Safety-related attrition – why?

1. Lack of early detection of safety signals

2. Lack of detection of safety hazards preclinically

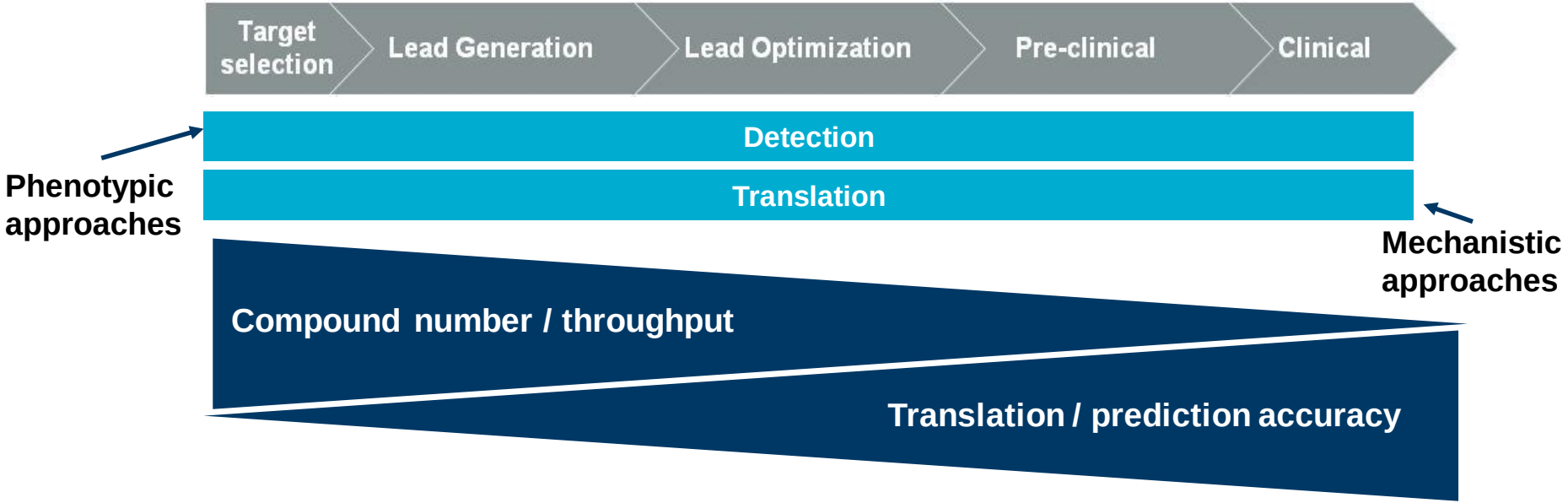
3. Lack of confidence / knowledge / precision in preclinical-clinical translation including healthy vs diseased states

Improved methods and models can impact:
- Detection
- Translation

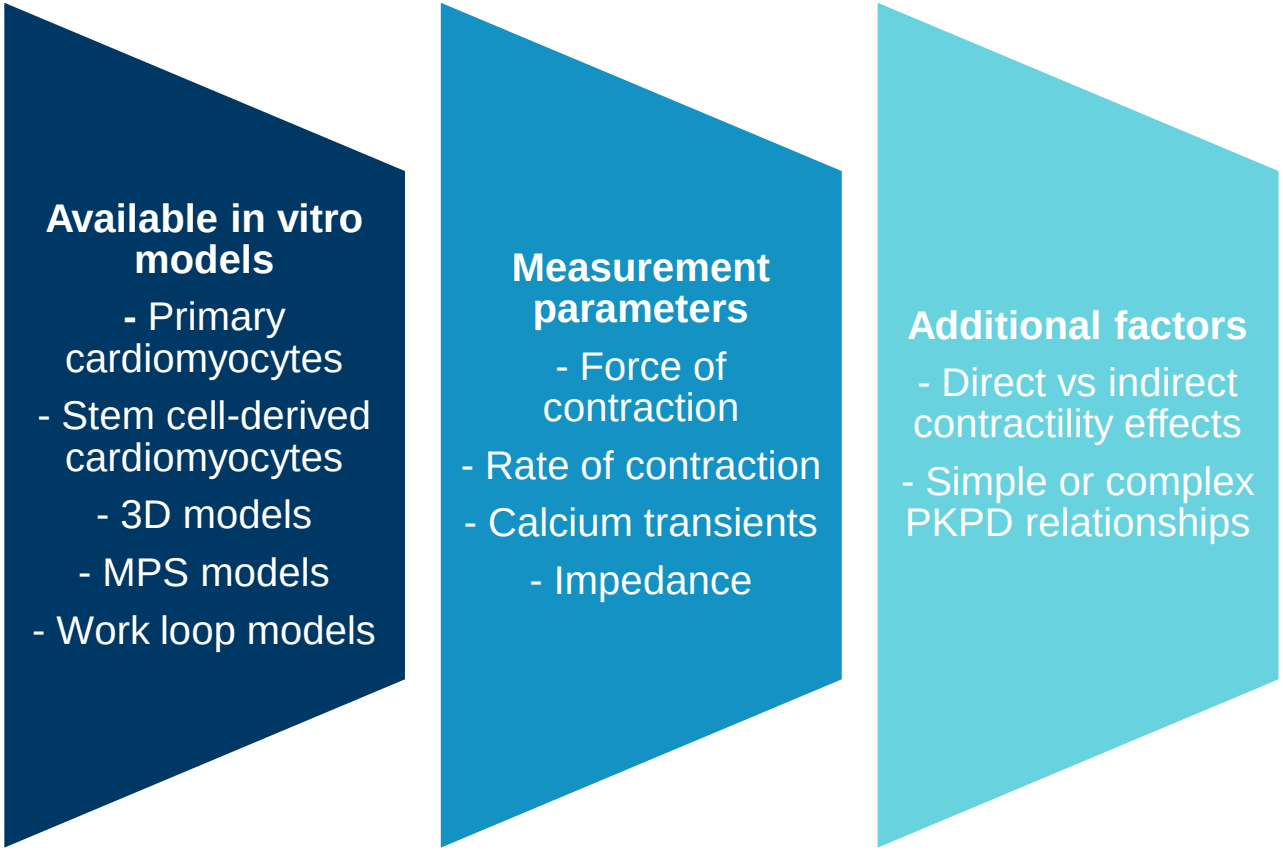
→

Ideally one model system would address all areas, however the requirements and stage of implementation in drug discovery differ

Appropriate model selection drivers

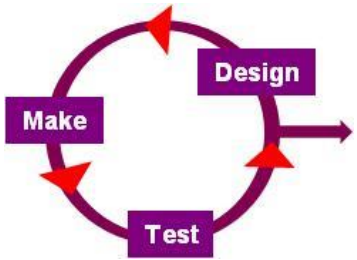


Cardiac contractility in vitro models: considerations



Cardiac contractility: Detection

Lead Generation: Aim: identify liabilities early, assay characteristics



This means in vitro assays ideally should:

- Be predictive of risk in man
- Drive understanding of SAR
- Have a short loop-time
- Be low cost
- Avoid use of animal tissues
- Capable of screening 100's of compounds weekly

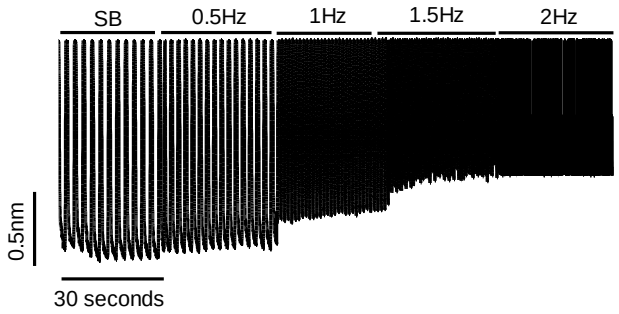
High throughput

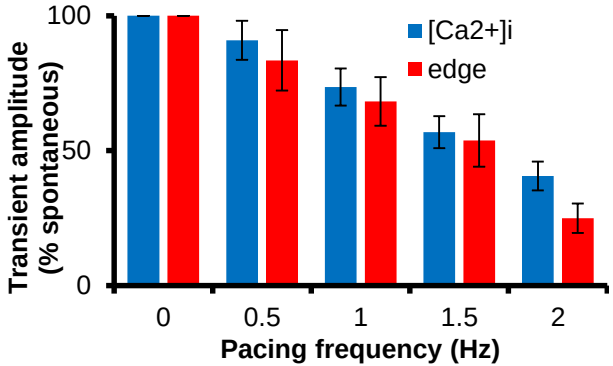
- **Impedance**
- **Calcium transients**
- **Cell movement**

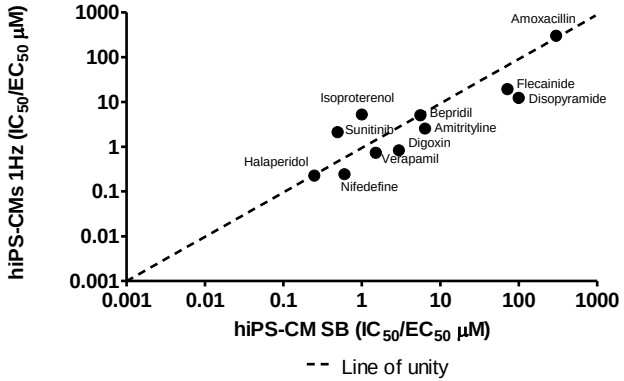
Available models

- Stem cell-derived cardiomyocytes
- Human cardiomyocytes

Cardiac contractility: Detection hiPS-CMs





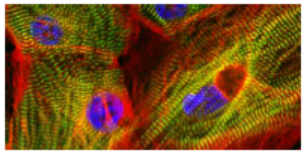


- hiPS-CMs can be electrically paced and contraction and calcium transients recorded
- Display a negative force frequency relationship
- Similar pharmacology under spontaneous beating and at 1Hz.

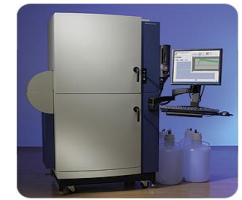
Pointon et al .Toxicological Sciences (2015) 144 (2): 227-237

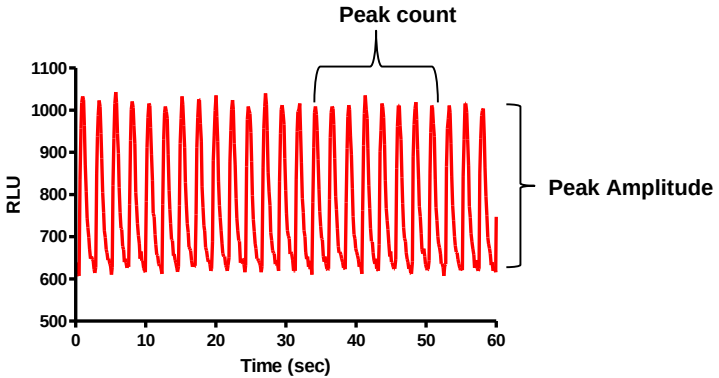
Cardiac contractility: Detection
Calcium transients

hiPS-CMs



FLIPR Jellyfish





Peak count – measures the number of calcium transients over a period of time.
Peak amplitude – measures the mean peak calcium transient as a % of resting calcium flux.

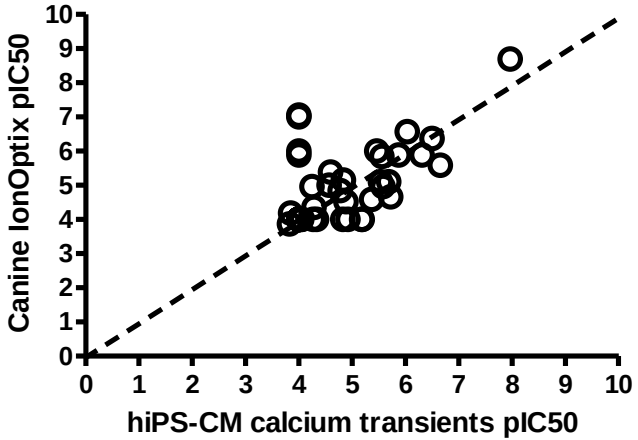
Monitoring the frequency and amplitude of iPS-CM calcium transients produces an integrated readout of cardiac function influenced by multiple cellular-factors

Pointon et al .Toxicological Sciences (2015) 144 (2): 227-237

Cardiac contractility: Detection Calcium transients

- Pharmacological validation using 50 reference compounds with known inotropic effects.
- Predicts direct, acute effects on contractility (upstream of the myofilaments).
- However doesn't discriminate between positive and negative inotropes – use to identify risk (detection)
- Overall good correlation to cardiac contractility in primary canine myocytes (low throughput assay).
- Suitable for positioning early in discovery.

Parameter	Definition	Score
Sensitivity	ability to detect true positive compounds	87%
Specificity	ability to detect true negative compounds	70%



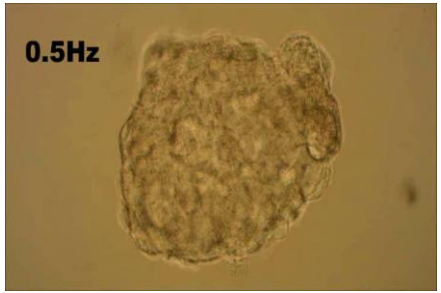
Pointon et al .Toxicological Sciences (2015) 144 (2): 227-237

3D cardiac microtissues: Detection

3D cardiac microtissues (hiPS-CMs, cardiac endothelial cells and cardiac fibroblasts) are able to overcome some of the disadvantages of hiPS-CM monolayer approaches.

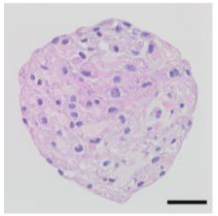
Functional assessment

- Typical contraction transients
- Spontaneous beat rate of 62 ± 24 beats/minute
- Maintained synchronized contraction transients following stimulation at 1, 2 and 3 Hz

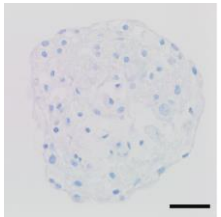


Morphological assessment

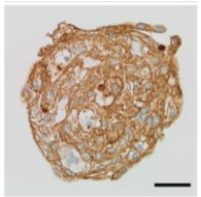
- Lack of apoptotic/hypoxic core
- All cell constituents present and proportionally appropriate



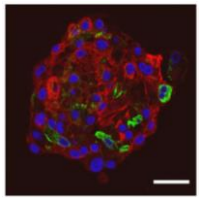
H&E



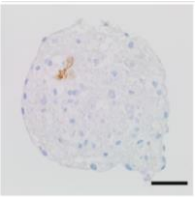
CC3



α-actinin

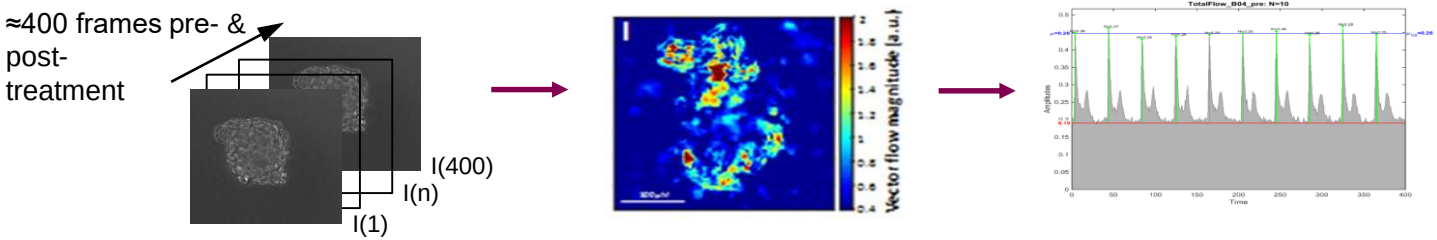


cTnI, Vimentin

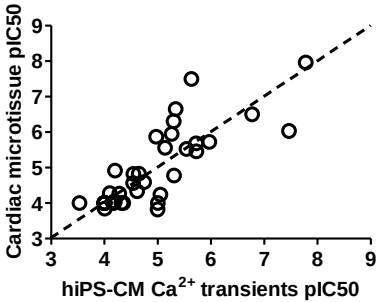


CD31

3D cardiac microtissues: Detection



Parameter	Definition	
Sensitivity	Ability to detect true positive compounds	93%
Specificity	Ability to detect true negative compounds	83%



- Good predictivity.
- Able to distinguish between negative and positive inotropes.
- Applicable in early discovery to detect direct acute inotropic agents.

Pointon et al 2017, Tox Sci 155(2):444-457

Cardiac contractility: translation

- 2D and 3D models are applicable to the detection of changes in cardiac contractility early during drug discovery while chemical choice is available.
    - Primary role: flag changes to direct chemistry away from a liability, if unsuccessful provide a flag to front load additional (*in vivo*) studies / monitoring
- The opportunity - how do we use *in vitro* models to move beyond detection to allow us to develop quantitative human translational understanding

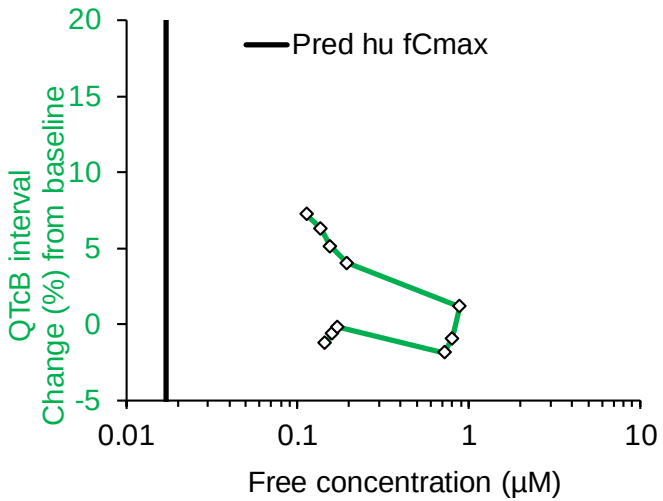
Cardiac contractility: moving beyond detection

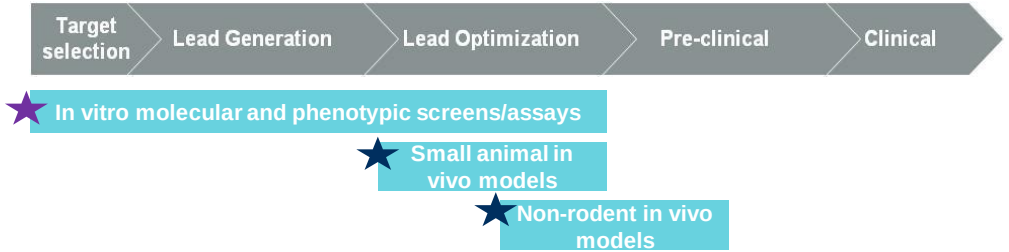
What are the challenges in developing in vitro approaches to deliver in quantitative translational understanding:

- Ability to model both simple and complex temporal PKPD relationships in vitro
- Mechanistic understanding and integrated physiology
- Knowledge and incorporation of patient specific factors

Improved:
- Risk assessment
- Translation

Cardiac contractility: moving beyond detection PKPD relationships





★ Simple concentration-response temporal relationships detected

★ Simple and complex concentration-response temporal relationships detected

Improved models and methods to detect complex PKPD relationships in vitro

What current advanced in vitro technologies potential could be applied?

- Cardiac loops[6]
- Patterning and flow[4,5]
- Matrix supports[1]
- Mechanical and electrical support[2]
- Microfluidic and perfusion systems[3]

1. Nat Methods. 2013 Aug;10(8):781-7.
2. Nat Comms. 7, 10312
3. Expert Opin Drug Discov. 2015 Mar;10(3):231-44
4. Biomaterials, 60, 20-30
5. Sci. Rep. **9,** 5:8883
6. Current Pharmaceutical Biotechnology 2013, 14:4-11

Could models with improved integrated physiology (healthy and disease) improve translation?

Cardiac contractility: moving beyond detection
Mechanistic understanding

Approaches to develop mechanistic understanding

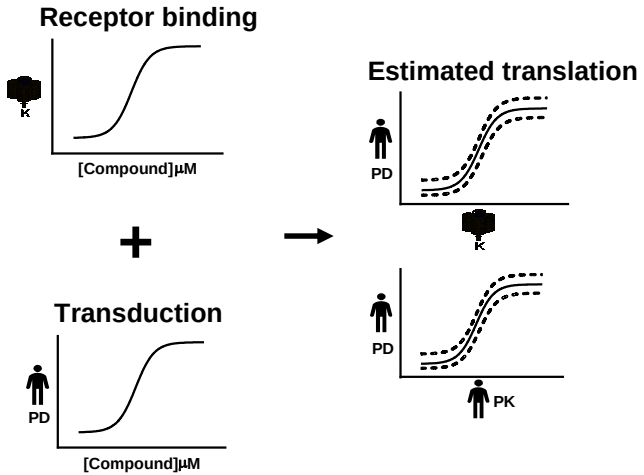
Text mining

Off-target profiling
Target class specific
- Radioligand binding
- KiNativ

Broad profiling
Agnostic to target class
- Chemoproteomics
- Proteomics
- Metabolomics
- Transcriptomics

Hypothesis generation aiding mechanistic understanding

Approach to develop quantitative translation



Mechanistic understanding simplifies quantitative translation.

Cardiac contractility: moving beyond detection
Patient centric considerations

Heart failure

Cancer Chemother Pharmacol. 2014 Mar;73(3):539-49. doi: 10.1007/s00280-014-2380-5. Epub 2014 Jan 22.

Phase I dose-escalation study of AZD7762, a checkpoint kinase inhibitor, in combination with gemcitabine in US patients with advanced solid tumors.

Sausville E[1], Lorusso P, Carducci M, Carter J, Quinn MF, Malburg L, Azad N, Cosgrove D, Knight R, Barker P, Zabludoff S, Agbo F, Oakes P, Senderowicz A.

Renal failure

J Am Soc Nephrol 14: 90–97, 2003

Effect of Chronic Renal Failure on Cardiac Contractile Function, Calcium Cycling, and Gene Expression of Proteins Important for Calcium Homeostasis in the Rat

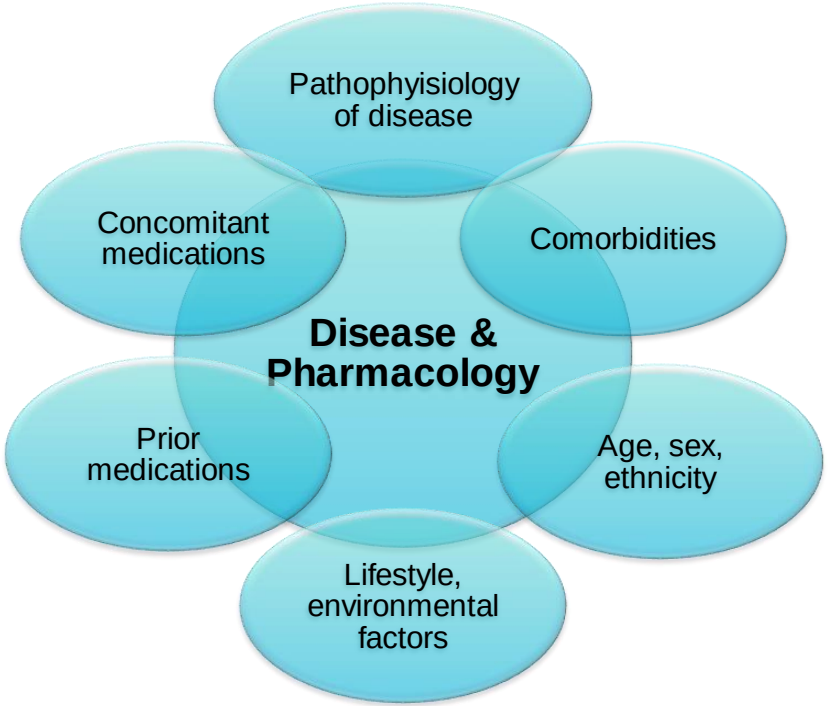
DAVID KENNEDY, EIAD OMRAN, SANKARIDRUG M. PERIYASAMY, JALAA NADOOR, ANUMEET PRIYADARSHI, JAMES C. WILLEY DEEPAK MALHOTRA, ZIJIAN XIE, and JOSEPH I. SHAPIRO
Departments of Medicine and Pharmacology, Medical College of Ohio, Toledo, Ohio.

Oncology

Am Heart J. 1999 Jul;138(1 Pt 1):78-86.

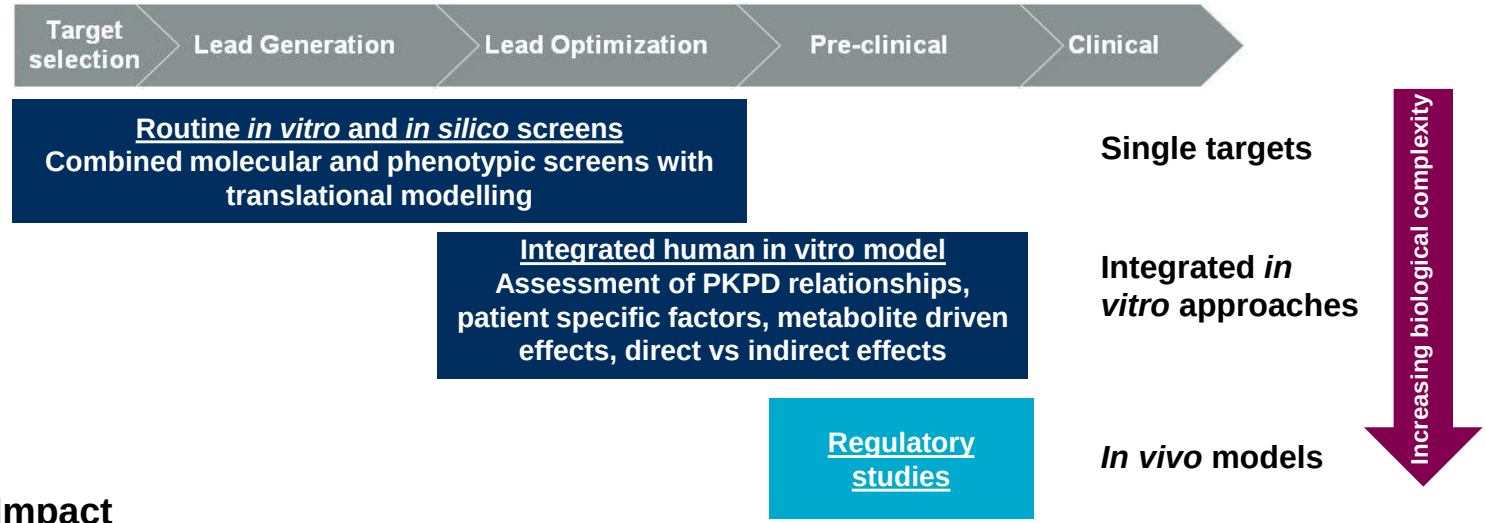
Continuous intravenous dobutamine is associated with an increased risk of death in patients with advanced heart failure: insights from the Flolan International Randomized Survival Trial (FIRST).

O'Connor CM[1], Gattis WA, Uretsky BF, Adams KF Jr, McNulty SE, Grossman SH, McKenna WJ, Zannad F, Swedberg K, Gheorghiade M, Califf RM.



Incorporation of patient specific factors would increase confidence in translation

What could the future holistic strategy for assessment of cardiac contractility look like



Impact

- Opportunity to develop quantitative translational understanding when combined with systems pharmacology modelling improving translation
- Human relevant risk assessment
- Reduction in animal use

Summary

- Model requirements/ expectations differ depending on stage of implementation in drug discovery.

- To improve quantatitive translational understanding we need to move away from focusing of detection of liabilities to mechanistic and PKPD understanding and incorporation of patient specific factors.

Acknowledgments

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