MAP4K4 mediates human cardiac muscle cell death: Preserving viability and function in human pluripotent stem cell-derived cardiomyocytes

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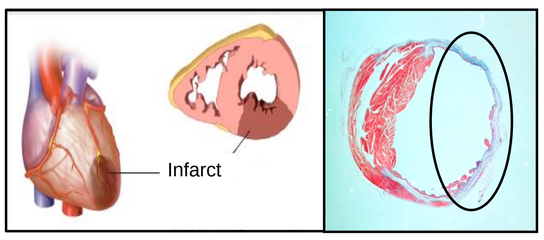
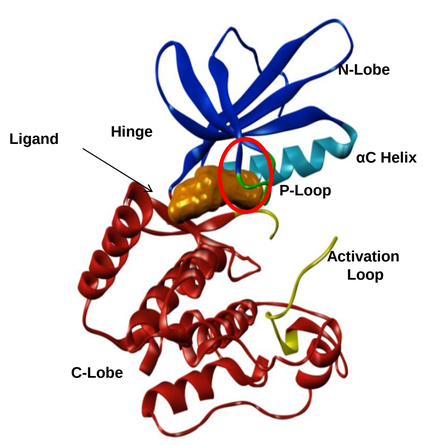
Overview

The Unmet Need

- Heart disease is a paramount cause of global death & disability.
- Cardiac muscle death is a component of both acute ischemic injury & chronic heart failure.
- Each 5% increase in infarct size increases the risk of heart failure & 1 year all-cause mortality by 20%.
- Current treatments do not directly benefit the endangered cardiomyocyte.
- Therapeutic progress has been hampered by lack of pre-clinical human validation.



The Target

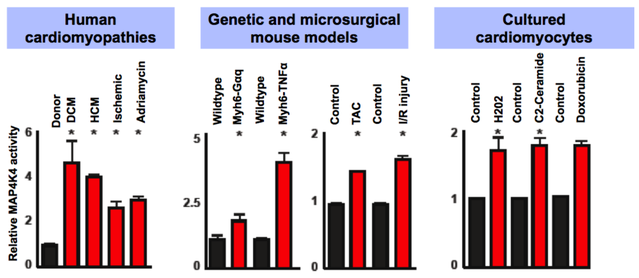


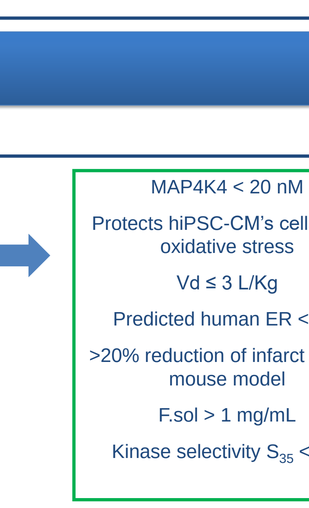
- Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4 (MAP4K4) is a serine-threonine kinase which activates the JNK signalling pathway.
- MAP4K4 has rare "folded" confirmation of P-Loop motif.
- Selective inhibitors were identified *via* virtual screen, taking advantage of this feature.

The Opportunity: Inhibiting MAP4K4 to Reduce Cardiac Cell Death

MAP4K4 is **activated**

- ✓ in diverse **human cardiomyopathies**
- ✓ in response to apoptotic triggers in **genetic** & **microsurgical mouse models**
- ✓ by oxidative stress & the anti-cancer drug, Doxorubicin, in **cultured rat cardiomyocytes**



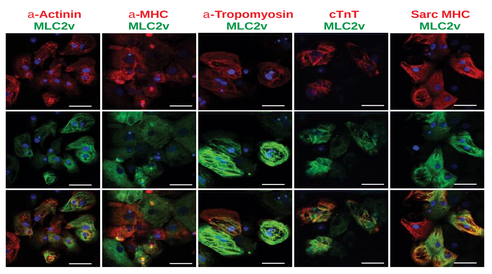


- Potent shRNAs conferred protection against H_2O_2.
- Gene silencing strongly suggests a requirement for endogenous MAP4K4 in human cardiac cell death.

The Competitive Advantage: Relevant Human Pre-clinical Model

iCell & CorV.4U cardiomyocytes derived from Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CM's):

- ✓ Express cardiac cell markers
- ✓ Spontaneously beat
- ✓ Expression of clinically relevant protein MAP4K4

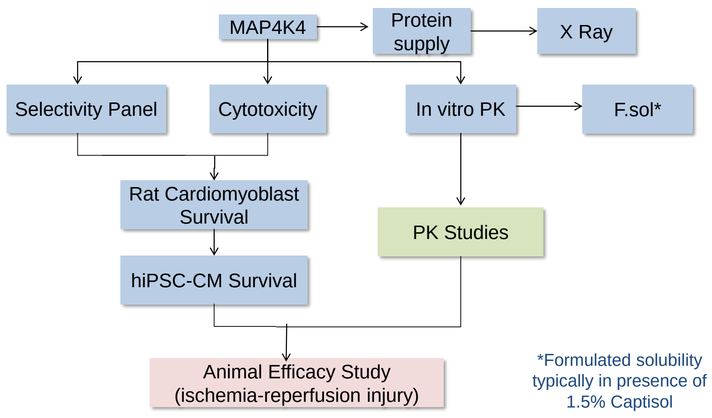


Hit-to-Lead Optimisation

Project Criteria

- To maximise benefit of a MAP4K4 inhibitor, rapid onset is required following myocardial infarction.
- We believe this is best achieved by i.v. bolus followed by maintenance infusion dose.
- In order to achieve this, high aqueous solubility is paramount.

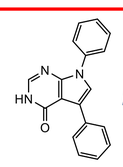
Screening Cascade



*Formulated solubility typically in presence of 1.5% Captisol

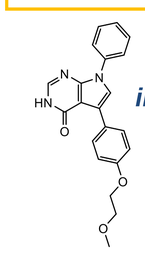
Compound Optimisation

DMX-4640
MAP4K4 40 nM
Mouse Cl > liver blood flow
Aq sol < 1 µg/mL
Kinase selectivity S_{35} < 0.1



Initial Hit

DMX-5804
MAP4K4 3 nM
Mouse Cl 33 ml/min/Kg
F.sol ~ 100 µg/mL
Kinase selectivity S_{35} < 0.1



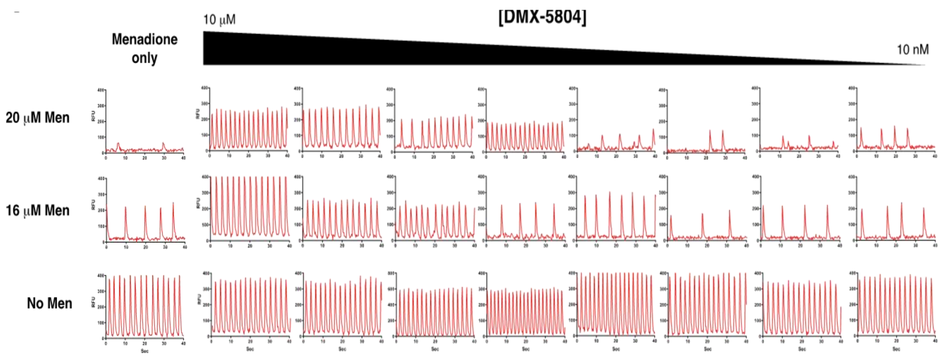
***in vivo* PoC**

MAP4K4 < 20 nM
Protects hiPSC-CM's cells from oxidative stress
Vd ≤ 3 L/Kg
Predicted human ER < 0.5
>20% reduction of infarct size in mouse model
F.sol > 1 mg/mL
Kinase selectivity S_{35} < 0.1

Pre-clinical Candidates

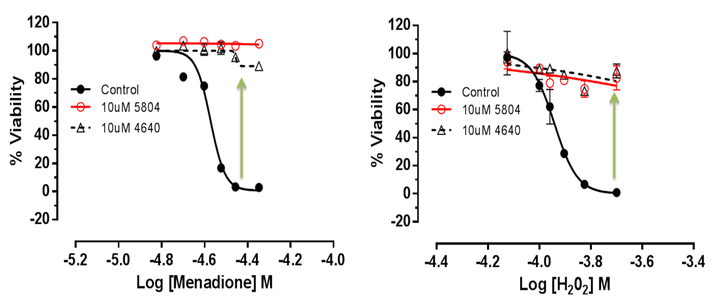
Target Validation

MAP4K4 Inhibition Protects Function of hPSC-CM's

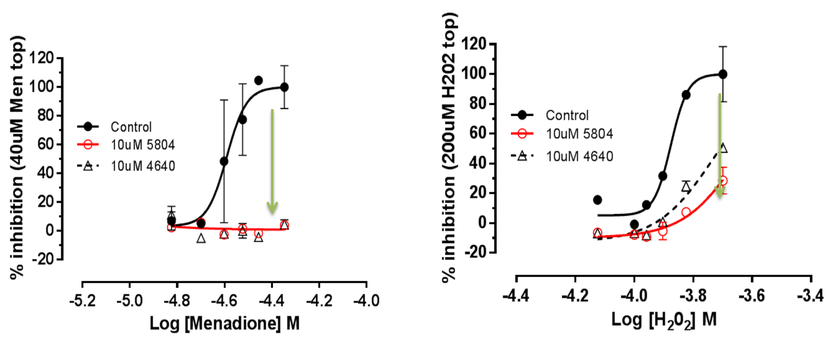


- Calcium sensing fluorescent dye reports on cardiomyocyte beating frequency & amplitude.
- Sub-lethal doses of Menadione affect cardiomyocyte function.
- DMX-5804 dose-dependently protects cardiomyocyte function from Menadione stress.
- Compound showed no toxic effect on cardiomyocyte function.

MAP4K4 Inhibition Confers Protection in hPSC-CMs & Reduces Cardiac Troponin I Release



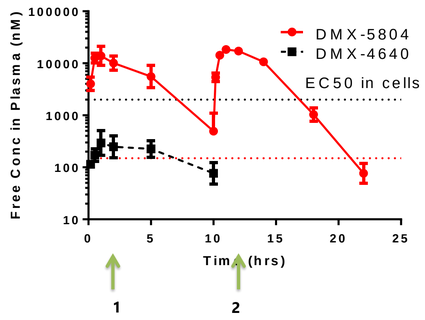
- DMX-5804 confers 100% protection against 2 oxidative stress signals in CorV.4U cells.
- Compounds showed no toxic effect on cardiomyocyte viability.



- Cardiac Troponin release is a clinical biomarker for myocardial infarction. Clinical read-out is Area-Under-the-Curve (AUC)
- Cardiac Troponin I can be measured *in vitro* using AlphaLISA technology. Time course in hPSC-CMs indicates 24 hr as optimal
- **DMX-5804 reduced cardiac Troponin I release.**

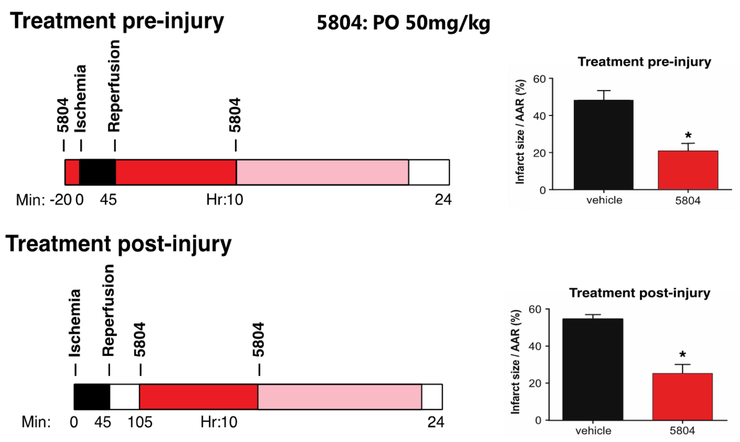
Mouse Efficacy Model

Po PK 50mg/Kg (twice dosing)



- Initial PoC carried out in mice using 50 mpk oral dose of DMX-5804.
- Exposure in excess of anticipated EC_{50} from cellular protection assays.
- *In vivo* MAP4K4 engagement confirmed using KiNativ probes.

- 2 different efficacy studies were carried out with the treatment post-injury having greater clinical relevance.
- **In both cases, ischemia-reperfusion injury in mice was reduced by >50%.**



Summary

- MAP4K4 is activated in failing human hearts & relevant rodent models.
- DMX-5804, a novel potent, highly-selective, small-molecule inhibitor of MAP4K4 was identified for PoC studies.
- DMX-5804 rescues cell survival & function in hiPSC-CMs & reduces ischemia-reperfusion injury in mice by >50%.
- **MAP4K4 is validated target for suppression of human cardiac cell death.**
- **This research shows the importance of hiPSC-CMs in drug development to enhance cardiomyocyte survival & function.**

About Domainex

Domainex is a fully integrated drug discovery CRO based in the UK. If you would like to learn more about applying our drug-discovery platform to your targets, please contact:
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