NLK, a pivotal kinase in cardiac disease: successful fragment expansion of ATP competitive inhibitors in the absence of crystallographic data

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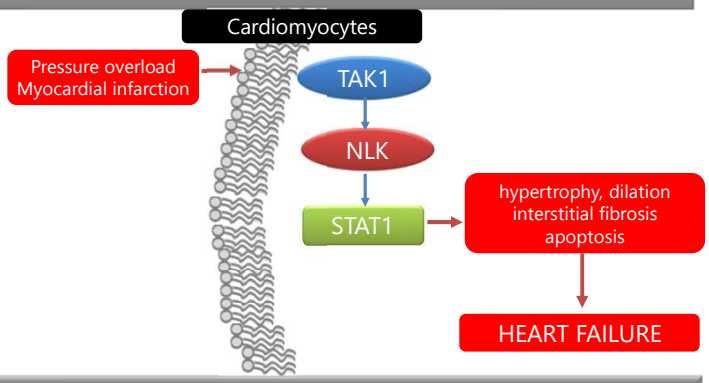
Introduction

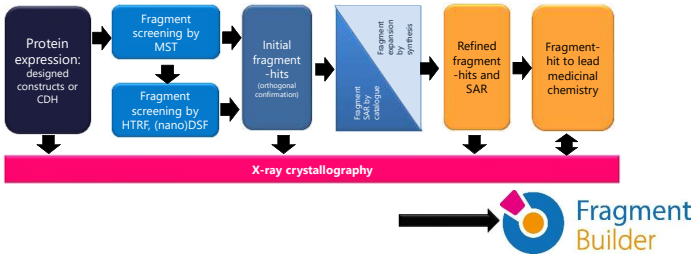
- Cardiovascular disease is the largest globally cause of death
- Its prevalence will increase as the population ages, offsetting gains in age-adjusted mortality
- Human cardiac muscle cell death is the hallmark of myocardial infarction
- Roughly 25% of heart attack patients develop heart failure within one year
- There are minimal effective therapeutics and a high failure rate in clinical trials which is a result of limited preclinical validation before embarking on human trials.
- Recent publication of MAP4K4 as potential new therapeutic for preventing heart attack damage has demonstrated the importance of a targeted approach to this unmet need (Schneider et al., 2019)
- Nemo-Like Kinase (NLK) has been shown to be a pivotal kinase in mouse models of cardiac disease (Molkentin et al. 2016)
- NLK is a Serine/threonine protein kinase
- It is a highly divergent, atypical member of the Mitogen-activated protein kinase (MAPK) group, lacking most features characteristic of most mitogen-activated protein kinases.
- There is no published crystal structure or patented inhibitors to-date.
- Literature president in achieving selectivity by targeting inhibitory sites allosteric to the ATP binding site with closest family member ERK



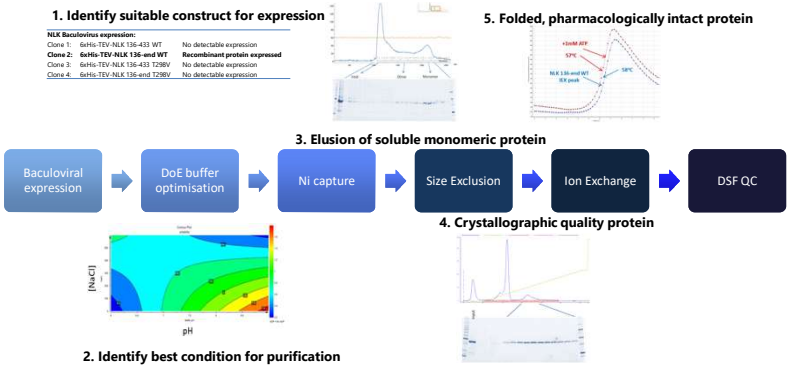
FBDD at Domainex

Domainex have a modular, rapid, and cost-effective FBDD platform called ***FragmentBuilder***

In collaboration with Prof Michael Schneider and with ICiC funding Domainex accelerated NLK through *FragmentBuilder* to identify novel and exciting hit molecules to form the basis of a drug discovery program for cardioprotection therapeutics, inhibiting this novel target

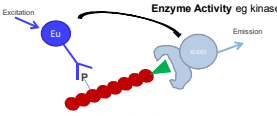


1. Successful expression and purification of NLK



2. Activity assay for fragment screening

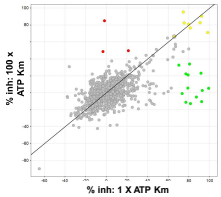
- HTRF (homogeneous time-resolved FRET) assay to measure phosphorylation of a generic kinase substrate by purified NLK
- Advantages of HTRF for fragment screening
  - Highly sensitive to molecular interactions
  - Amenable to high DMSO concentration
  - Low false positive rate
- Can be used to differentiate between ATP competitive, non-competitive and un-competitive inhibitors
- **No fragments or proprietary inhibitors are available for NLK**

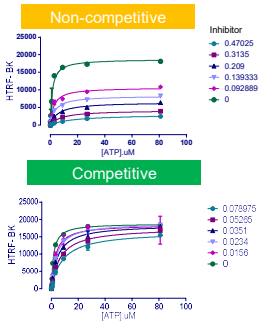


- Biotinylated-peptide substrate
- Phospho-antibody conjugated to Europium
- Streptavidin acceptor beads
- Red-shifted

3. Fragment screening and validation

Target & modality: Competitive, un- and non-competitive inhibitors of ATP
Assay format: HTRF activity assay screening at [ATP] = 1 X and 100 X apparent K_m
Fragment Library: Domainex 1000 fragment library screened at 1mM
Hit selection: > 50% inhibition at 1mM, no interference with technology
Confirmation: DSF and MST

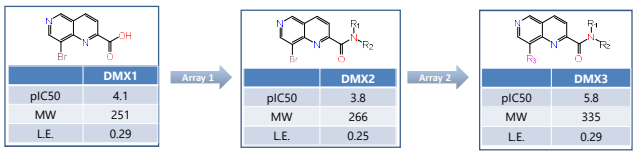




Red: ATP-dependant but not competitive (Uncompetitive)
Yellow: ATP-Independent and not competitive (Non-competitive)
Green: ATP-dependant and competitive (ATP competitive inhibitors)

Clustering of hits: Shift in % inhibition followed by mechanism of action studies using Michaelis –Menten Kinetics

4. Fragment expansion and SAR



- The protein did not crystalise despite extensive testing of different conditions
- In the absence of structural information, fragment expansion efforts were focused on the ATP-competitive compounds
- Domainex fragment library is designed to incorporate functional groups to enable rapid array chemistry so SAR can be assessed and potency improved
- >50-fold increase in potency was achieved with just **2 rounds** of medicinal chemistry (each array contained **~15 compounds**) using only the docking of fragments into a homology model of the target to guide compound design.
- **DMX3** is suitable for cellular proof of concept experiments, and will be tested by Domainex in a cardioprotection assay
- However, compounds that bind outside the ATP pocket require structural information for efficient expansion
- Domainex are exploring alternative approaches to obtain structural information

Summary

- NLK is a therapeutically-relevant target for cardioprotection
- Domainex has:
  - successfully expressed NLK for FBDD
  - identified unique validated fragments available that either (i) bind at the ATP binding site, or (ii) bind at other sites than ATP.
  - designed low µM potency inhibitors by expansion of ATP-competitive fragments without structural information with a minimal number of iterations; such compounds may be suitable for PoC in cells
- The protein used for bioassay was not amenable to crystallographic studies; therefore we are currently investigating different structural approaches such as cryo-EM, or alternative construct selection using Domainex CDH technology, to gain data that can drive SBDD

Contacts and References

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about applying our drug-discovery platform to other targets, please contact: **ray.boffey@domainex.co.uk, www.domainex.co.uk, #DomainexDelivers!**

1. Ruijie Liu, Hadi Khalil, Suh-Chin J. Lin, Michelle A. Sargent, Allen J. York, and Jeffery D. Molkentin, (2016) "Nemo-Like Kinase (NLK) Is a Pathological Signaling Effector in the Mouse Heart" PLoS one
2. Lorna R. Fiedler, Kathryn Chapman, Min Xie, et al., Catherine Tralau-Stewart,Trevor Perrior, Michael D. Schneider (2019) "MAP4K4 Inhibition Promotes Survival of Human Stem Cell-Derived Cardiomyocytes and Reduces Infarct Size In Vivo" Cell Stem Cell