IN SILICO TECHNOLOGIES IN DEVELOPMENT OF INHIBITORS FOR
MST1 – THE KEY REGULATOR OF BETA CELL APOPTOSIS AND
DYSFUNCTION IN DIABETES

PRINCIPLE INVESTIGATORS IN THE CONSORTIUM:

- Dr. Elena Fedorova, VVS Lab, Russia
- Dr. Xiang-Guo Li, Turku University Hospital and University of Turku, Finland
- Professor Mark Johnson, Åbo Akademi University, Finland
- Karina Patz, St. Petersburg Chemical Pharmaceutical Academy, Russia

Moscow, 2016
Diabetes in numbers

THE ECONOMIC COSTS OF DIABETES MAY EXCEED THE REVENUES FROM ECONOMIC GROWTH ALL COUNTRIES IN THE WORLD

REAL PEOPLE

Percentages and predictions can mask the enormity of the diabetes problem. Large numbers of people with diabetes are unaware they have the disease because they have not been diagnosed (shown as the shaded edge in the country bubbles). The imperative for public-health professionals is to diagnose and treat people as soon as possible.


346 M people worldwide have diabetes. More than 80% of diabetes deaths occur in low and middle-income countries, according to the WHO.
### MST1 or Mammalian Sterile 20-like-1 (or STK4)
- Serine/threonine protein kinase
- Key component of Hippo complex → Pro-apoptotic
- Phosphorylates/inactivates PDX1 in the beta cells

### PDX1 or Pancreatic and Duodenal Homeobox-1
- Insulin promoter factor 1
- Needed for beta cell maturation
- MST1 phosphorylation → Impaired insulin secretion

### Block MST1 with inhibitors to treat diabetes

---

**MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes**

Amin Ardestani¹, Federico Paroni¹,6, Zahra Azizi¹,6, Supreet Kaur¹,6, Vrushali Khobragade¹, Ting Yuan¹, Thomas Frogne², Wufan Tao³, Jose Oberholzer⁴, Francois Pattou⁵, Julie Kerr Conte⁵ & Kathrin Maedler¹
PDX1 involvement in diabetes
MST1 kinase regulates PDX1

- PDX1 activates multiple genes associated with glucose metabolism, including insulin, and PDX1 mutations are associated with development of diabetes in childhood.

MST1 is a key mediator of beta cell apoptosis and dysfunction, and MST1 depletion completely restores normoglycemia and prevents diabetes progression.

The goals

1. Stop phosphorylation of the PDX1 protein
   - WHY?
     - Leads to beta cell apoptosis
   - Improving outcome in diabetes

2. Produce an in vivo tracer of beta cell health
   - WHY?
     - Signal level of tissue damage
     - Report on efficacy of therapy
Solution

**Objective:** To restore the function of β-cells

**Bivalent Inhibitor**

**SOLUTION:** Prototype of inhibitor

**Small Molecular Compounds**

**Peptide Sequences**
A lot of kinases...
MST1 kinase domain structure

- Bi-lobed kinase domain: N- and C-terminal lobes
- ATP/Mg<sup>2+</sup> complex is bound in a cleft
- Activation loop (or A-loop) phosphorylated → Activity

http://wsklab.wustl.edu/~shude/bioPRS/farure22/phospho.png
Kinase domain sequence
Obvious attack on ATP binding site
Obvious, but very challenging

Have excellent three-dimensional structural data on kinase domains, but only the homeobox domain of PDX1 is known at this time

- L/I and L/C variation extends across many kinase families
- High selectivity nearly impossible?
- Seek other strategies, too
Ensemble-based virtual screening

**Receptor Ensemble**
- NMR or X-ray crystallography
- 3D receptor structure
- Homology modelling
- Molecular Dynamics (MD) simulations
- Snapshots extracted
- Manual selection
- Cavity detection
- Systematic reduction

**Ligand Ensemble**
- Commercial databases
- Organic synthesis
- Virtual screening
- NIB screening
- Docking
- Protein-ligand complexes
- MD/MM-GBSA
- Hierarchical screen
- Docking scoring

**Post-processing**
**GUSAR - GENERAL UNRESTRICTED STRUCTURE ACTIVITY RELATIONSHIPS**

**Staurosporine Derivative**

\[ \text{pred} \text{IC}_{50} (\text{Staurosporine}) = 2.5 \times 10^{-6} \]

inhibit the target by 40%

**Pred \text{IC}_{50}**

- \[ 5.6 \times 10^{-7} \] (45% of inhibition MST1)
- \[ 8 \times 10^{-7} \] (69% of inhibition MST1)
- \[ 1.1 \times 10^{-9} \]

**In vitro test ADP-Glo™ assay kits for MST1**

- \[ 7 \times 10^{-6} \] \( R^2 = 0.77 \)
- \[ 63 \] \( R^2 = 0.85 \)
Positron emission tomography
Precursors and linkers for radiolabeling of peptides

1. 

2. 

3. 

4. 

Inhibitors for diabetes

ATP binding site

MSTI

PDX1 binding site
Diabetes Susceptibility Genes Pdx1 and Clec16a Function in a Pathway Regulating Mitophagy in β-Cells.

Soleimanpour SA¹, Ferrari AM², Raum JC², Groff DN², Yang J², Kaufman BA³, Stoffers DA⁴.

- Restoring Clec16a led to normalisation of mitochondrial trafficking
Acknowledgments

Prof. Vladimir Poroikov for supply of GUSAR software as well as his consultation

Prof. Anne Roivainen for translational PET imaging and disease modeling, and her role to advice in study design of the prepared compounds.

Prof. Juhani Knuuti for guide this project from clinical aspects, as well as preclinical studies.

Funding gratefully acknowledged from the University of Turku, Sigrid Juselius Foundation, Academy of Finland

Thank you for your attention!
Fedorova Elena
elena.vic.fedorova@gmail.com