Discovery and Development of MAP Kinase Kinase 4 (MKK4)-Inhibitors for treatment of acute and chronic liver diseases
Treatment of Liver Disease –

An Unmet Medical Need:

• Liver disease represents a major global health problem (estimated prevalence of liver cirrhosis average ~1% in all populations)
• >1.5 Mio people die of liver failure and liver disease will continue to rise and double within the next 20 years
• Despite possible eradication of HCV, liver disease will continue to increase due to fatty liver disease: 20% of all Americans are affected by NAFLD which progresses into NASH and liver failure
Pathogenesis of acute and chronic liver failure:

Background

Modified from: Nature Reviews Cancer
A Direct In Vivo RNAi Screen Identifies MKK4 as a Key Regulator of Liver Regeneration

Torsten Wuestefeld,1,2,3 Marina Pesic,2,3 Ramona Rudalska,2,3 Daniel Dauch,2,3 Thomas Longerich,5 Tae-Won Kang,2,3 Tetyana Yevsa,1 Florian Heinzmann,2,3 Lisa Hoenicke,2,3 Anja Hohmeyer,1,3 Anna Potapova,2 Ina Rittelmeier,1,6 Michael Jarek,2 Robert Geffers,2 Maren Scharfe,2 Frank Klawonn,2 Peter Schirmacher,5 Nisar P. Malek,4 Michael Ott,1,6 Alfred Nordheim,7 Arndt Vogel,1 Michael P. Manns,1 and Lars Zender1,2,3,*

1Department of Gastroenterology, Hepatology & Endocrinology, Medical School Hannover, 30625 Hannover, Germany
2Helmholtz Centre for Infection Research (HZI), 38124 Braunschweig, Germany
3Division of translational Gastrointestinal Oncology, Department of Internal Medicine I, University of Tübingen, 72076 Tübingen, Germany
4Department of Internal Medicine I, University of Tübingen, 72076 Tübingen, Germany
5Institute of Pathology, University Hospital Heidelberg, 69120 Heidelberg, Germany
6Twincore Centre for Experimental and Clinical Infection Research, Feodor-Lynen Strasse 7, 30625 Hannover, Germany
7Department of Molecular Biology, Interfaculty Institute for Cell Biology, University of Tübingen, 72076 Tübingen, Germany

*Correspondence: lars.zender@med.uni-tuebingen.de
http://dx.doi.org/10.1016/j.cell.2013.03.026
A Direct In Vivo RNAi Screen Identifies MKK4 as a Key Regulator of Liver Regeneration

... provides experimental evidence, that RNAi-mediated suppression of MKK4 expression ....

- Unlocks endogenous regenerative capacity of hepatocytes (via compensatory upregulation of MKK7 and JNK1-dependent activation of ATF2 and ELK1) in diseased livers...
- increases the robustness of hepatocytes in vivo and in vitro, and
- reduces fibrosis and increases liver function in chronic liver damage
Science/Technology

Explored
Experimental Models:

**Acute damage/failure:**
- CD95 induced acute liver failure
- 2/3 hepatectomy

**Chronic damage/failure:**
- CCL4 induced CLF
- FAH-/- deficiency
- Hepatocyte primary culture/transplantation
- ASH, NASH

Willenbring & Grompe, Cell 2013

=> **M KK4: one therapeutic target for the treatment of a broad range of acute and chronic liver diseases**
Effect of MKK4 knockdown on the robustness of hepatocytes in exp. acute liver failure:

- Acute liver injury
- Liver failure
- Increased apoptosis
- High level of serum TNFα
- Low survival

Wüstefeld et al., Cell, 2013
Increased robustness and EdU incorporation of primary shMKK4 expressing human hepatocytes
Safety:
MKK4 knockdown does not increase tumor development

A

12 months after injection

Bright

12 months after injection

α-FAH

GFP

H & E

B

shMKK4

0.5±0.5

shNC

0.6±0.6

C

AML12

Tumor volume (mm)

D

BNL CL.2

Tumor volume (mm)

E

BNL 1NG A.2

Tumor volume (mm)
Generation of inducible shMKK4 transgenic mice
Mimicking Systemic Drug Action via Conditional ubiquitous shRNA-expression

Caggs-rtTA3-TRE.shMKK4.A

No overt toxicity after in a 12 month observation period
Selectivity: Therapeutic effect of MKK4 knock-down depends on MKK7, JNK1, ATF2 and ELK1 induction
Effect of a small molecule MKK4 inhibitor (first optimized hit) on survival in exp. acute liver failure:

Representative macroscopic images of livers treated with carrier or the optimized hit.
Strategy, Concept & Business Plan

Strategy:

Discovery and Development of small molecule inhibitors of MKK4:

1. Virtual Screening
   • Analysis of MKK4 crystal structure
   • Binding models established
   • Virtual screening of several libraries
   • Promising Hits optimized

Current Status: 2 Hit-to-Lead programs ongoing
Strategy, Concept & Business Plan

Strategy:

Discovery and Development of small molecule inhibitors of MKK4:

2. High-Throughput Library Screening
   - Fluorescence-based assay in 384 well plates developed and validated
   - Library with >70,000 compounds tested
   - 4 scaffolds selected for further characterization
   - Selectivity testing performed

Current Status: Hit-to-lead program ongoing
Strategy, Concept & Business Plan

Concept: Semi-Virtual Setting

CMOs:
- Molecular biology
- In vitro/in vivo pharmacology
- Animal PK
- Clinical pharmacology

HepaRegeniX

CRO:
- Design Hit-to-lead-to-candidate program
- Chemical synthesis
- Bioanalytical support
- In vitro metabolism
Strategy, Concept & Business Plan

Plan:

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