Sorafenib and Liver Regeneration in Rats

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Presentation is based on following articles:

1. **The natural history of liver regeneration in rats: Description of an animal model for liver regeneration studies.**
   - Published in *International Journal of Surgery*, 2013
   - Kasper Jarlhelt Andersen, Anders Riegels Knudsen, Anne-Sofie Kannerup, Hideki Sasanuma, Jens Randel Nyengaard, Stephen Hamilton-Dutoit, Erland J. Erlandsen, Bo Jørgensen, Frank Viborg Mortensen.
   - DOI: 10.1016/j.ijsu.2013.07.009

2. **Sorafenib inhibits liver regeneration in rats.**
   - Published in *HPB*, 2013
   - Kasper Jarlhelt Andersen, Anders Riegels Knudsen, Anne-Sofie Kannerup, Jens Randel Nyengaard, Stephen Hamilton-Dutoit, Morten Ladekarl, Frank Viborg Mortensen.
   - DOI: 10.1111/hpb.12068

3. **Postoperative but not preoperative treatment with Sorafenib inhibits liver regeneration in rats.**
   - Accepted for publication in *Journal of Surgical Research*, Primo 2014
   - Kasper Jarlhelt Andersen, Anders Riegels Knudsen, Anne-Sofie Kannerup, Jens Randel Nyengaard, Stephen Hamilton-Dutoit, Morten Ladekarl, Frank Viborg Mortensen.
Liver regeneration

- Animal studies can be used as an accelerated model of human liver regeneration.

- Many different methods to create liver regeneration in experimental animal studies:
  - Alcohol, surgery, carbon tetrachloride, galactosamine, AAF, etc.

- Surgical approach: 70 % partial hepatectomy

- Well established and used since described in 1931 by Higgins and Anderson

- However, there is no consensus of how and when to evaluate liver regeneration in animal studies.
Proliferation after liver resection in a healthy liver: Mature hepatocytes

Mature healthy hepatocytes can de-/trans-differentiate

Pathways involved in cell proliferation:

Signal transduction.
Source: en.wikipedia.org
Study I: Natural history

- Aims:
  - To investigate the natural history of liver regeneration in healthy rats.
  - To develop and test new design-based stereological methods in evaluation of liver regeneration.

- Hypothesis:
  - Liver regeneration in healthy rats, is a fast process completed after approximately one week.
Natural history: Study design

- 64 Wistar rats of 200 g.
- 70% partial hepatectomy.

Main endpoints:
- Mortality.
- Liver and body weight.
- Regeneration Rate.
- Stereological evaluation.
- BTR (Liver function).
The rats were randomised for euthanisation post operative day 1 – 8.
Natural history: Results

- Mortality:
  - 5 animals (8%) died prior to evaluation.
    - Ileus (1).
    - Suture gnawing/Intestinal perforation (1).
    - Unidentifiable causes (3).
Natural history: Results

![Body Weight Graph](image_url)
Natural history: Results

Liver Weight

Liver weight (g)

Day of Sacrifice

Before Res. Day 0
After Res. Day 0
POD 1
POD 2
POD 3
POD 4
POD 5
POD 6
POD 7
POD 8
Natural history: Results

Regeneration Rate

Day of Sacrifice

Before Res. Day 0
After Res. Day 0
POD 1
POD 2
POD 3
POD 4
POD 5
POD 6
POD 7
POD 8

0
20
40
60
80
100
120
140
Regeneration Rate
Natural history: Results

ALAT

Concentration (U/L)

Day of Sacrifice

POD 1  POD 2  POD 3  POD 4  POD 5  POD 6  POD 7  POD 8
Natural history: Results

![Graph showing BTR over Day of Sacrifice](image-url)
Hepatocyte proliferation: KI-67 staining

- Design-based stereological method for an unbiased estimation of cell proliferation.
- SURS sections immunostained for the nuclear KI-67 antigen.
- Coloring cells not in G-0 phase.
- Independent of tissue deformation.
- Standardized method not prone to inter and intra-observer variability.
Natural history: Minimal hepatocyte proliferation
Natural history: Maximal hepatocyte proliferation
Natural history: Results

Hepatocyte Proliferation

Day of Sacrifice

Ki-67 Ratio [pos cell profiles/area], mm²
Natural history: Conclusions

- The natural history of liver regeneration in rats is a dynamic process with a maximum regenerative speed on PODs 1-4 and is practically complete on POD 8.
Study II and III: Sorafenib and liver regeneration

- Sorafenib = Nexavar = BAY 43-9006.

- A multi tyrosine kinase inhibitor:
  - Anti-proliferative.
  - Anti-angiogenic.

- Approved for HCC.

- Halts tumour growth but is not curative.

- Question: Can one expect normal liver regeneration during treatment with sorafenib?
Hepatocellular carcinoma: Oncogenic pathways in cells

Neoplastic Diseases Reviews. ‘New Molecular Biomarkers...’ CancerLink.ru
Hepatocellular carcinoma: Angiogenic pathways

Sorafenib: Mechanism of action

Sorafenib targets both tumour-cell proliferation and angiogenesis in vitro

**In preclinical models**

Adapted from Wilhelm et al 2004

Nexavar Mechanism of Action - Nexavar


Next: The RAS/RAF/MEK/ERK Pathway and HCC

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Study II: Perioperative treatment with sorafenib

Aim:
- To investigate the impact of multikinase inhibition by sorafenib on liver regeneration in healthy rats.

Hypothesis:
- Multikinase inhibition with Sorafenib, 15 mg/kg/day, in a peri-operative setting inhibits liver regeneration in healthy rats, following 70% hepatectomy.
Sorafenib peri-operative: Study design

- 60 Wistar Rats of 200 g:
  - 30 Sorafenib.
  - 30 NACL.

- Oral gavage 14 days prior to resection and until sacrifice.

- Sorafenib dose:
  - Dissolution of tablet in NACL.
  - 15 mg/kg daily.
  - Rats were given 0.75 ml each day.

- Resection consisted of 70 % PHx.
Sorafenib administration: Oral gavage
Sorafenib peri-operative: Flow chart

- The rats were randomised for euthanisation post operative day 2, 4 or 8.

3 groups of 10 rats
- 2 weeks Sorafenib
- 70 % PHx
- Continued Sorafenib
- Euthanization POD 2, 4 or 8

3 groups of 10 rats
- 2 weeks placebo
- 70 % PHx
- Continued placebo
- Euthanization POD 2, 4 or 8
Sorafenib peri-operative: Results

- Mortality:
  - Seven animals (5%) died prior to evaluation:
    - Suture gnawing/Intestinal perforation (4).
    - Unidentifiable causes (3).
  - No difference in mortality between groups
Sorafenib peri-operative: Results

Body Weight

Body Weight (g)

Day of Sacrifice

Before Res. Day 0  After Res. Day 0  POD 2  POD 4  POD 6

*p<0.001  *p<0.001  *p=0.162  *p=0.025  *p<0.001

Sorafenib  Placebo
Sorafenib peri-operative: Results
Sorafenib peri-operative: Results
Sorafenib peri-operative: Results

![Graph showing proliferation - Ki 67 stereology](image)

- Day of Sacrifice:
  - POD 2
  - POD 4
  - POD 6

- Proliferation - Ki 67 stereology

- Analysis with *N=2
  - Sorafenib
  - Placebo
Sorafenib Peri-operative: Conclusions

- Multikinase inhibition with Sorafenib, in a peri-operative setting does not increase mortality in rats undergoing liver resection.

- Multikinase inhibition with Sorafenib, in a peri-operative setting inhibits liver regeneration, judged by liver weight, regeneration rate, and hepatocyte proliferation.
Study III: Pre- or post-operative treatment with sorafenib

Aim:
To investigate the impact of pre- or post-operative treatment with multikinase inhibitor, sorafenib, on liver regeneration in healthy rats.

Hypothesis:
Multikinase inhibition with Sorafenib, 15 mg/kg/day, in a pre- or post-operative setting inhibits liver regeneration in healthy rats, following 70% hepatectomy.
Sorafenib pre- or post-operative: Study design

- 120 Wistar Rats of 200 g
  - 60 Sorafenib
  - 60 NACL

- Oral gavage 14 days prior to resection or from resection and until sacrifice

- Sorafenib dose:
  - Dissolution of tablet in NACL
  - 15 mg/kg daily
  - Rats were given 0,75 ml each day

- Resection consisted of 70 % partial hepatectomy
Sorafenib pre- or post-operative: Flow chart

3 groups of 10 rats
- 2 weeks Sorafenib
- 70 % PHx
- No treatment
- Sacrificed POD 2, 4 or 8

3 groups of 10 rats
- 2 weeks placebo
- 70 % PHx
- No treatment
- Sacrificed POD 2, 4 or 8

3 groups of 10 rats
- 2 weeks No treatment
- 70 % PHx
- Sorafenib
- Sacrificed POD 2, 4 or 8

3 groups of 10 rats
- 2 weeks No treatment
- 70 % PHx
- Placebo
- Sacrificed POD 2, 4 or 8
Sorafenib pre- or post-operative: Results

- Mortality:
  - Eleven animals (9%) died following surgery.
    - Suture gnawing/Intestinal perforation (6).
    - Unidentifiable causes (5).
  - No difference in mortality between groups.
Sorafenib pre- or post-operative: Post-op
Sorafenib pre- or post-operative: Post-op
Sorafenib pre- or post-operative: Post-op
Sorafenib pre- or post-operative: Post-op
Sorafenib pre- or post-operative: Pre-op
Sorafenib pre- or post-operative: Pre-op
Sorafenib pre- or post-operative: Pre-op
Sorafenib pre- or post-operative: Pre-op
Sorafenib pre- or post-operative: Conclusions

- Multikinase inhibition with Sorafenib, in a pre- or post-operative setting *does not increase mortality* in rats undergoing liver resection.

- Multikinase inhibition with Sorafenib, in a post-operative setting *inhibits liver regeneration*, judged by liver weight, regeneration rate, and hepatocyte proliferation.

- Multikinase inhibition with Sorafenib, in a pre-operative setting *does not inhibit liver regeneration*, judged by liver weight, regeneration rate, and hepatocyte proliferation.
Acknowledgements

Danish Cancer Society
Aarhus University
Aarhus University Hospital
Viborg Regional Hospital

Thank you for your attention
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