Innovative inhibitors of Notch trafficking and signalling for the treatment of cancer
The Notch Signaling Pathway

- **Regulates:**
  - patterns of gene expression
  - cell differentiation
  - binary cell fate choice
  - maintenance of stem cell populations

- **Function:**
  - Embryonic Development
  - Adult Self-Renewing Organs

Kopan R & Ilagan M, Cell 2009 | Vol 137 Issue 2 pp.216-233
Abnormal Notch Signaling and Cancer

**Oncogenic activity of Notch**
- Notch active → Progenitor → Differentiated Cell
- Notch inactive

**Tumor suppressor activity of Notch**
- Notch inactive → Progenitor → Differentiated Cell
- Notch active

**Notch Misregulation**
- Blocks Differentiation
- Promotes Survival
- Increases Proliferation
Estimated new Cancer cases in the US 2014

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>27%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>8%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>7%</td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>5%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>5%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>4%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>3%</td>
</tr>
<tr>
<td>All other sites</td>
<td>20%</td>
</tr>
<tr>
<td>855,220</td>
<td>810,320</td>
</tr>
</tbody>
</table>

| Breast       | 29%         |
| Lung & bronchus | 13%       |
| Colon & rectum | 8%         |
| Uterine corpus  | 6%          |
| Thyroid       | 6%          |
| Non-Hodgkin lymphoma | 4%       |
| Melanoma of skin | 4%       |
| Kidney & renal pelvis | 3%       |
| Pancreas      | 3%          |
| Leukemia      | 3%          |
| All other sites | 21%       |

Hyperactive Notch Signaling

Notch is a rational target in Cancer Therapy

Many steps of intervention are discussed for Notch signaling:

- γ-Secretase Inhibitors (GSIs)*
- ADAM-Inhibitors (ASIs)
- Endocytosis inhibitors
- Transcriptional Complex Inhibitors
- Antibodies
- Synthetic peptides
- Trafficking inhibitors

*Currently in Clinical Trials
  e.g. BMS-906024, RO04929097, MK-0752, and PF3084014

Kopan R & Ilagan M, Cell 2009 | Vol 137 Issue 2 pp.216-233
Screening for new Notch Inhibitors

Notch∆E-EGFP

PM → γ-sec. Notch∆E-EGFP → NICD-EGFP → nucleus

DMSO

Fli-06

Krämer et al, Nat Chem Biol 2013
FLI-06 suppresses Notch signaling

**Inhibition of Notch in vitro in C2C12 cells**
Luciferase activity

**Inhibition of Notch in vitro in A549 lung cancer cells**
Notch target gene

**Inhibition of Notch in vivo in Zebrafish**
Notch target gene

![Graphs and images showing inhibition of Notch signaling](image.png)
Inhibition of Notch-dependent tumor cells

**FLI-06 on DND-41**

**viable cell count**

- **DMSO**
- **10µM FLI-06**
- **1µM FLI-06**

Day 0 Day 1 Day 2 Day 3 Day 4
**FLI-06 and inhibition of SASP**

**Senescence Associated Secretory Phenotype**

- Cytokines
- Chemokines
- Proteases
- Growth Factors

DNA Damage

- Ribosomal Stress
- Spindle Stress
- Chromatin Distortion
- Telomere Dysfunction
- Oxidative Stress

Senescent cell
FLI-06 and inhibition of SASP

SASP and cancer

in vitro SASP model

Naylor RM et al, Clinical Pharmacology & Therapeutics 2013 | Vol 93 | Issue 1 | pp. 105-116
Development Challenge

Compound Profiling

- Toxicity Data (in vitro and in vivo)
- SAR and Derivatization
- Target Identification
  - Pharmacokinetics, Pharmacodynamics

Clinical relevance

- Cancer, SASP
- Cancer Therapy?
- Disease Model (Zebrafish, Mouse, Nothobranchius)
- Favorable outcome?

Activity profiling

- ER Ultrastructure
- Further study of cellular effects
R&D Status

Strength
- The compound targets the Notch pathway and presumably reduces side-effects
- Secretion Inhibition may reduce Relapse

Weakness
- Early Phase Development
- Mode of action not yet clear

Opportunities
- A novel unanticipated mode of action is beneficial for cancer treatment
- Compound class already marketed as drugs

Threats
- As all Notch Inhibitors it lacks specificity
- No satisfactory outcome after closer investigation

Funding

Scientific Input

Manpower

Co-Development

Infrastructure

In-Licensing
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PAKT FÜR FORSCHUNG UND INNOVATION
LeibnizAgeNet: signalling pathways in age-related diseases

Thank you for your attention!
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Further Reading:
