This presentation:

• Introduction to technology
• Measuring Kinase Activities
• Biomarker research
• PamGene’s PamAcademy
Small introduction into Pamgene

• Founded in 2000, LSP funded, Akzo Nobel spin off.
• Tech and biomarker IPR portfolio
• Services & Partnering in R&D Life Sciences
• Unique proprietary array-based platform for kinases & nuclear receptors for:
  – Lead Identification and Lead Optimization,
  – (Pre) clinical translational research
  – Biomarker development in Food & Pharma research
• Supporting Compound Development (Food, Pharma)
  – Lead Optimization through selectivity profiling of kinase inhibitors & NR-Food-coregulator interactions.
  – Develop selective kinase & NR modulators in cells and tissues (xenograft, PDTX, patient material, solid tumor).
  – MOA differentiation of Kinase and NR ligands at a functional and molecular level in cell & tissue models.
  – Patient stratification via biomarker profiling
  – Human Response prediction via biomarker profile.
How did we started in Wageningen
How did we started

Bridge to new WUR collaborations in NH receptors: From reporter gene assays to coregulator binding assays

Estrogen Receptor α (ERα)
Alternative for the uterotrophic assay?

Wang et al. Alternatives to Animal Experimentation (ALTEX) (2013)
How did we started II

Alternatives for animal testing

Estrogen Receptor α (ERα)

Alternative for the uterotrophic assay?

In one series:
dose response curves and
EC50 values
for 155 coregulators determined
for 18 model compounds
for which data from
uterotrophic assay
are available

Wang et al. Alternatives to Animal Experimentation (ALTEX) (2013)
CAT Agro food: PamStation: nuclear receptors
From reporter gene assays to coregulator bindings assays
Alternatives for animal testing: Coregulator fingerprints for 14 estrogens

Hierarchical clustering

structurally related compounds cluster together compounds having an aromatic A-ring were separated from those with a cyclohexene A-ring

assay reflects structural similarity of ERα agonists, indicating a potential to achieve identification and classification of ERα endocrine disruptors

## MARCoNI Services

### MARCoNI Assays

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MARCoNI silver</th>
<th>MARCoNI gold</th>
<th>MARCoNI platinum</th>
<th>MARCoNI diamond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coregulators #</td>
<td>5</td>
<td>15</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Coregulator conc.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1-8</td>
</tr>
<tr>
<td>Nuclear receptor*</td>
<td>LBD</td>
<td>LBD</td>
<td>LBD, FL</td>
<td>LBD, FL, Lysates</td>
</tr>
<tr>
<td>Compound conc.</td>
<td>[1-3]</td>
<td>[1-3]</td>
<td>[1-3; IC$_{50}$]</td>
<td>[1-3; IC$_{50}$]</td>
</tr>
<tr>
<td>Price-structure</td>
<td>Per cmp + setup fee</td>
<td>Custom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHRs #</td>
<td>25+ nuclear receptors available</td>
<td>Advanced bioinformatics</td>
<td></td>
<td></td>
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</tbody>
</table>

### Selectivity profiling** (up to 20 NHRs)

- **Safety-Panel**
  - (AhR, CAR, PXR, PPARα, FXR, LXR, TR and RAR)
- **Metabolic-Panel**
  - (PPARα, 6/δ, γ, LXRs FXR)
- **Endocrine-Panel**
  - (ERα, PR, AR, GR, MR, VDR, TRs)

- Custom: ✓
- Single: ✓

*LBD = Ligand Binding Domain; FL- Full length NHR protein; Lysates = Nuclear Receptor extracts from cell lysates

**The NHR panels have not been defined yet.
Measuring Kinase Activity

Proteomics

PamChips

2D Arrays

Mass Spec

Western blot

Nuclear Hormone Receptor

Ligand
Cellular lysates

Recombinant kinase
Yeast
Cell based
Xenograft
Mice
Zebrafish
Clinical samples

Kinase

Serine Threonine

Clinical samples
Most important signaling enzymes phosphryl transferases
2% of human genome
~ 30% of proteome phosphorylated
518-534 kinases
Main pharmaceutical drug target
PKI research: biased!
Measuring kinase activity II

DNA → RNA → Protein

Genotype

"Classical" methods
“presence”

Activity/response

Activity based method

Gene expression

Missing: Technology bridging the gap between protein abundance and phenotype
Different peptides are immobilized on the array:
  Kinase application **144**
  NHR application **53/155**
Measuring Kinase Activity with the assistance of the CCD camera

Sample is pulsed back and forth through the array

Every spot on the array or line on the graph is representing a peptide sequence that is phosphorylated on the array.

Phosphorylation by Abl kinase

<table>
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<tr>
<th>Time (min)</th>
<th>Signal (AU)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
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<tr>
<td>15</td>
<td>150</td>
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<tr>
<td>20</td>
<td>200</td>
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<td>75</td>
<td>750</td>
</tr>
<tr>
<td>80</td>
<td>800</td>
</tr>
</tbody>
</table>

0 20 40 60 80

ATP

ADP

Kinase + P

---Y---------
Functional Proteomics on PamChip® arrays

Differences in Spots (peptides)

BioNavigator6

Patient Sample → Ex-vivo PamChip profiles

No Drug

Drug

Measuring Kinase Activity V

Functional Proteomics on PamChip® arrays
Towards kinase pathways

- From fingerprints to pathway
- Mechanism of action elucidation
Measuring Kinases: Cell based assays

- Rec Enzymes
- Cell line models

→ Animal models

→ Clinical Trials
Workflow cell lysate PamChip assay

“Lyse cells/tissue and profile”

1. Harvest cells
2. Spin down
3. Add lysis buffer
4. Lysis for 30’ on ice
5. Apply on PamChip (1-10 ug prot)
6. Run incubation protocol
7. Analyse phosphorylation profiles

Real time data
within 40 minutes

Fast versus western, or gene expression profiling
Mouse derived knock out T-cells

Purified mouse T-cells of WT and KO mice were stimulated with anti-CD3 for 0, 30, 60 or 120 minutes.

Unstimulated control samples were provided for WT and KO for each time point.

### Samples provided

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Protein (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0min</td>
<td>WT unstimulated</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>+ anti-CD3</td>
<td>1.4</td>
</tr>
<tr>
<td>30min</td>
<td>KO unstimulated</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>+ anti-CD3</td>
<td>1.1</td>
</tr>
<tr>
<td>60min</td>
<td>WT unstimulated</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>+ anti-CD3</td>
<td>1.2</td>
</tr>
<tr>
<td>120min</td>
<td>KO unstimulated</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>+ anti-CD3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

- Samples were run on STK PamChips
- Samples were run on PTK PamChips
Protein Tyrosine Kinase profiling in T-cells

Stimulation by anti-CD3 treatment and increased stimulation when combined with anti-CD28 in WT cells at 30 min.

144 phosphorylations

(no WB/FACS bias)
Measuring Kinases: Clinical Trials

Rec Enzymes
Cell line models

Animal models

Clinical Trials
Example:
Predicting Therapy Response in Rectal Cancer Patients

- Phase II clinical trial;
  - 67 patients treated with oxaliplatin and 5-FU in a Preoperative Chemoradiation (CRT) trial NCT00278694

- Goal:
  - Predict CRT prior to therapy using kinase activity profiling

- Conclusion:
  - 85% correct prediction

Kinases Analysis Workflow: how does it work

Type of chips

- PamChip 96 array
- PamChip 4 array

Type of PamStations

- PamStation®96
- PamStation®12

BioNavigator

- (controlling PamStation)
- (software for data analysis)
Test materials

Small amount of tissues & cells
Per array 1-10 µg total protein or 10,000 - 100,000 cells

Primary and cultured Cells (animal model and patient material)
- White blood cells, blood platelets, PBMCs, Bone marrow
- Primary cells e.g. liver
- Culture cells, adherant or suspension cells

Primary tumors and biopsies (animal model and patient material)
- Freshly frozen, archival tissues, up to 20 years old
- Tumor content >70% (>20% also used)
- Different tissues (FNA) e.g. lung, liver, breast, brain, prostate, skin, thyroid, CSF

Laboratory animals
- Zebrafish, rat, mouse tissue: xenograft tissue, pig, dog

Other
- Yeast, Plant
Biomarker Research with PamGene
Joining the future with PamGene I

Dedicated Drug/Food treatment & selection for patients

- Start treatment
- Response to food?
- Patient diagnosed
- Period the patient is treated with the drug/food

Inhibition profile of compound --> Response prediction of the man towards the drug
Joining the future PamGene III

Actual work flow of the routine clinical practice

Step 1
Human Sample
(5 minutes)

Step 2
Testing the sample of the patient with a drug/food
(30 minutes)

Step 3
Result of the patient automatic analyst
(5 minutes)
Take home message

- Dynamic **peptide microarrays**
- **Kinetics** → more knowledge
- **Kinase activity** (and nuclear **receptor function**) profiling of the full-length and fully decorated target proteins
- **Biomarker platform** for target and biomarker discovery and validation in **precision medicine**
- Drug specific profiles / biomarkers
- Bridging technology: abundance → activity → phenotype
- Translational platform: no platform changes in phase transitions
- Test inhibitory efficacy of drug or food *ex vivo*
  - to **predict clinical response**
Take home image

measurement

DNA abundance
RNA abundance
Protein abundance

analysis

interpretation

biomarkers
pathways
systems knowledge
Take home image (II)

DNA abundance
RNA abundance
Protein abundance
Target class activity

measurement

(Pre) Clinical Profile

analysis

interpretation

biomarkers
pathways
systems knowledge

Reversed Translational Research

Wageningen, January 14th 2014
We would like to successfully support your research needs
PamGene’s PamAcademy

How?

High sensitive measurements
Dedicated and proven software
Training programs in order to get people successful
Sharing 10 years of kinase activity expertise
Sharing knowledge also by using the PamCloud
Scientific publications
Share your research question
PamGene’s PamAcademy III

To continue your success we recommend

Create and handle samples according our protocol

Lyse the samples according to our protocol

Share your research problem, we have experience in creating scientific possibilities

Get trained with the PamAcademy

Use our powerful software tool to analyze loads of data
Explore next steps with CAT-AgroFood and PamGene:

• Aim to continue your research needs
• Support you with high tech proteomic tools
• Train you and validate your skills
• Together creating new collaborations