# Application of proteomics in drug development

# Outline

- Concept Overview
- Tissue proteomics for establishing drug targets
- Urinary proteomics for patient stratification and monitoring
- Animal Models
- Clinical Trials

- **Concept Overview**
- Tissue proteomics for establishing drug targets
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# Key issues

- **Proteome analysis enables diagnosis, prediction, and prognosis with unsurpassed accuracy.**
- **Improved diagnostics requires better therapeutic drugs to enable personalized medicine.**
- **Proteome analysis will inform about pathophysiology and ideal therapeutic targets.**

# Concept Overview

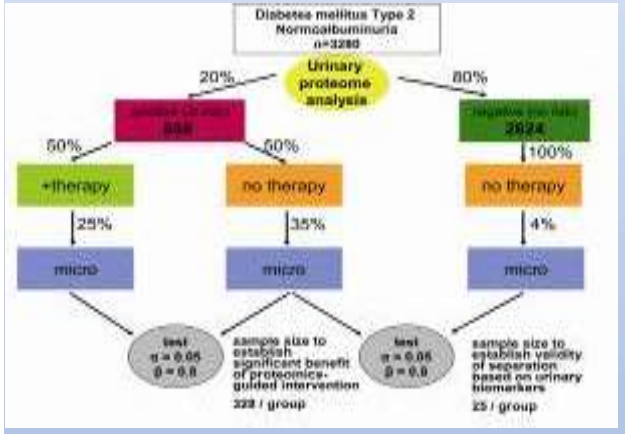
- **Proteins** are active key players in the cell driving normal and pathological processes.
- The aim is to **identify proteins responsible for disease-specific processes**, and then deliberately target them with drugs.
- To test the drugs, an appropriate **model system has to be selected,** as closest possible to the human disease at the molecular level.
- **Combinatorial Approach**: Therapeutic targets, monitoring and stratification biomarkers and optimal animal models, can support drug development.

## Biomarker discovery

## Systems Medicine

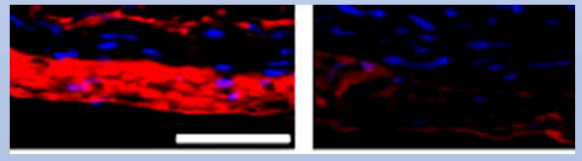
**Integration** of experimental and literature data

**Verification** of potential biomarkers and classifiers

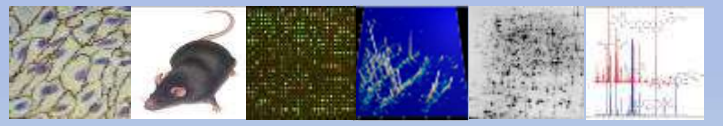



Biological Specimens

**In silico** prediction of molecular changes



**Verification** of predicted changes



Investigation of potential **therapeutic targets**

**Drug development**

Assessment in the relevant population in **study/trial**

## Application

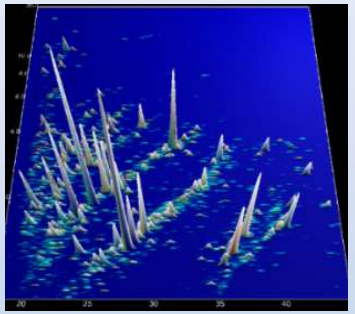
# Human proteome database

## Clinical data, Patients history

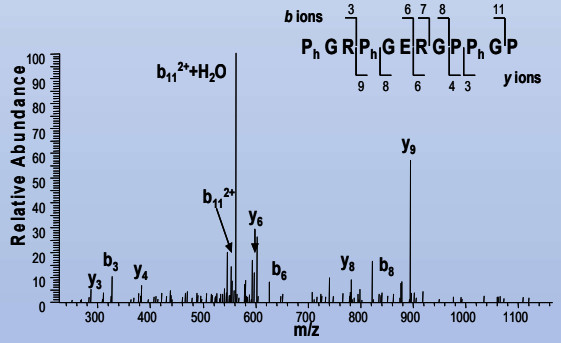
| |
|---|
| Age |
| Gender |
| Urinary albumin/creatinine |
| Cholesterole (mmol/l) |
| Creatinine (micromol/l) |

## Database

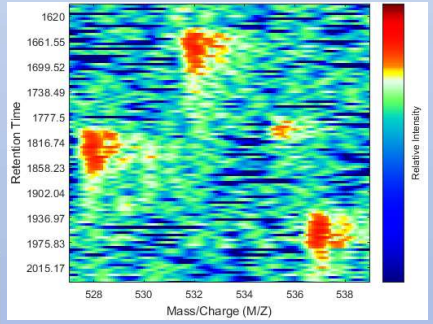
## Urinary proteomics data



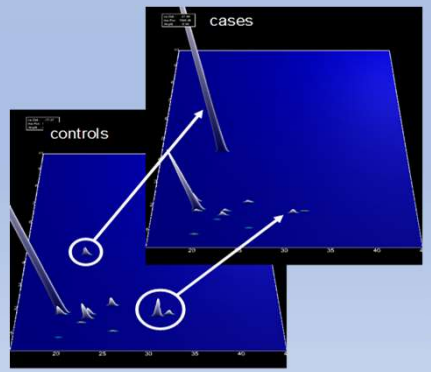
## Sequence information



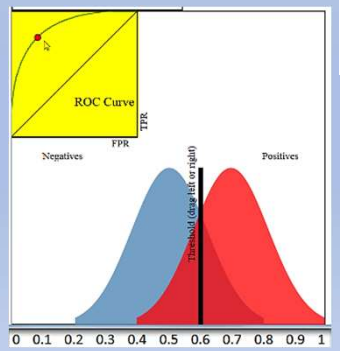

## Tissue proteomics data




## Biomarker selection




## Statistics



# Proteomics analysis: characteristics

## Tissue Proteomics

**Advantages:**

- Tissue is the site of disease initiation and progression
- Provides a direct link to pathophysiology
- Allows for understanding disease-associated mechanisms

**Limitations:**

- Limited sample size
- Restricted availability

## Urinary Proteomics

**Advantages:**

- Easily obtainable/ non-invasive
- Reflects systemic/peripheral disease associated changes
- Enables the identification of disease specific urinary profiling signatures (biomarkers)

**Limitations:**

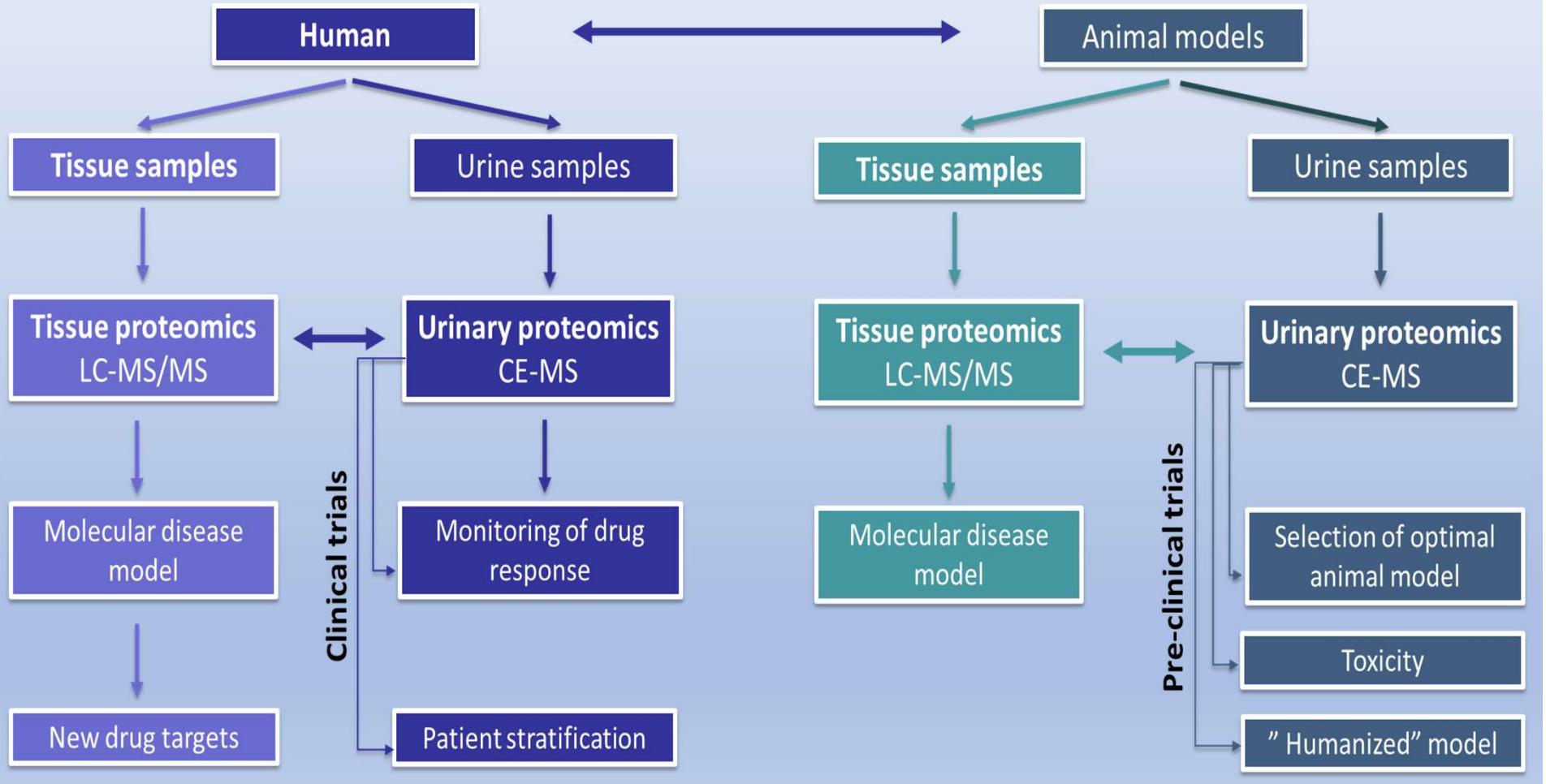
- No direct connection to disease etiology

**Complementarity: Data Cross-correlation**

## MOS' Combinatorial Approach Enables:

- Identification of potential targets for therapeutic intervention
- Selection of optimal animal models/ alignment to human disease (humanized models)
- Initial patient stratification/ pre-selection
- Monitoring of drug efficacy and off-side effects (organ toxicity)

# MOS' Workflow



# Areas of interest

- Coronary Artery Disease
- Heart Failure
- Chronic Kidney Disease

- Bladder Cancer
- Prostate Cancer

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- **Tissue proteomics for establishing drug targets**
- Urinary proteomics patient stratification and monitoring
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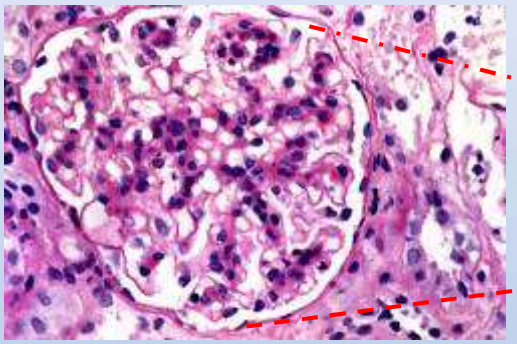
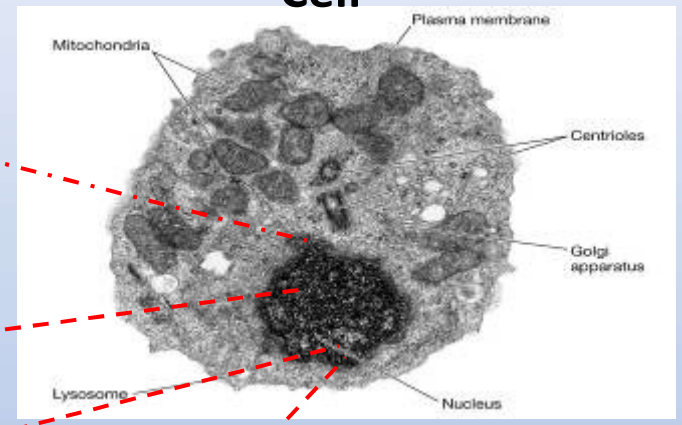
# Proteins generally are the targets for drugs

Drug

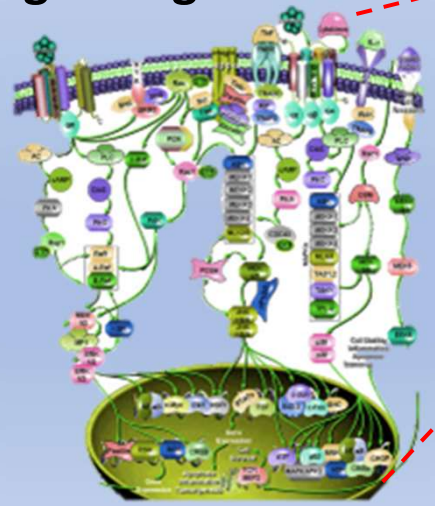
Tissue



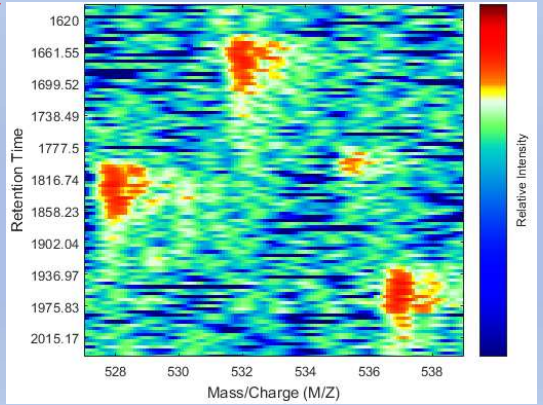
Cell




Signalling molecules (proteins)

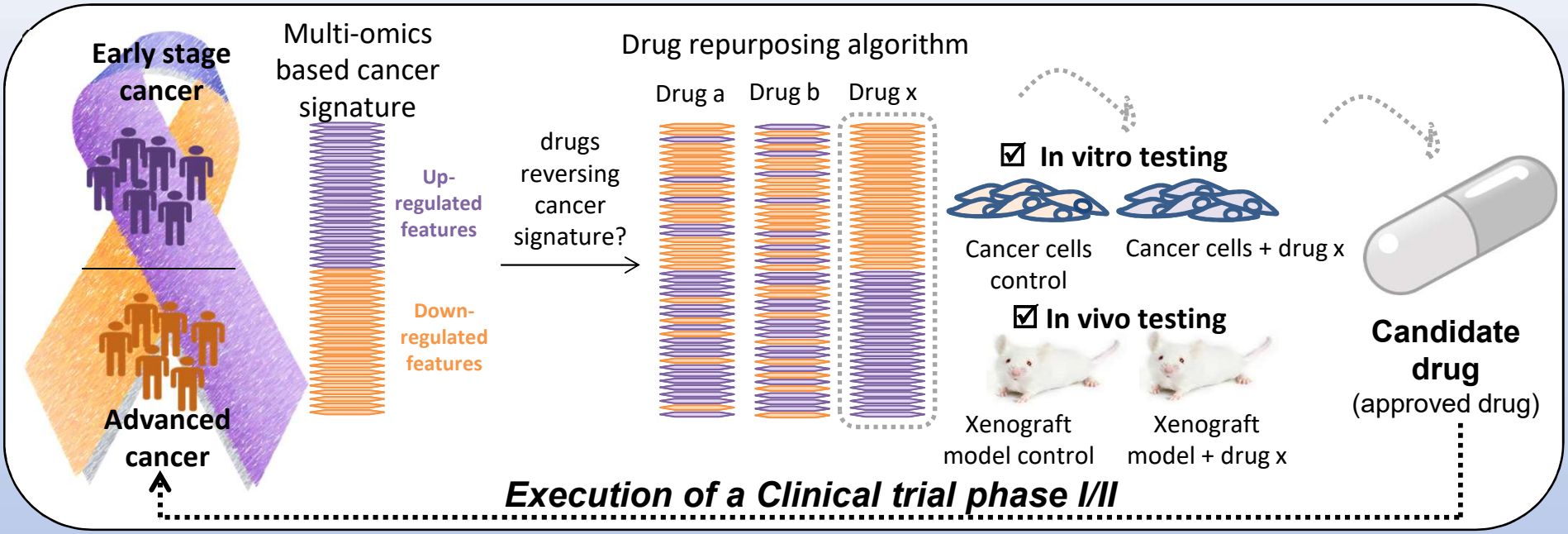


Tissue Proteome




Adapted from Mischak et al (2015) **Nephrology Dialysis Transplantation**

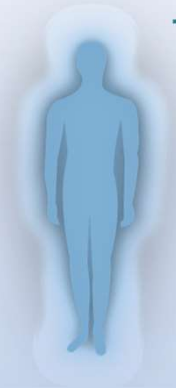
# MOS approach




1) **Molecular characterization of cancer progression though integration of high-throughput multi-omics data from patients via advanced computational tools (development of disease signature).**
2) **Application of an innovative AI-based pipeline to predict drugs having the potential to reverse disease progression.**
3) **Rationale selection of drug candidates in line with disease pathophysiology.**
4) **Testing of the candidate drugs in relevant preclinical model systems.**

# A completely novel all-in-one solution

## A patients-centered approach

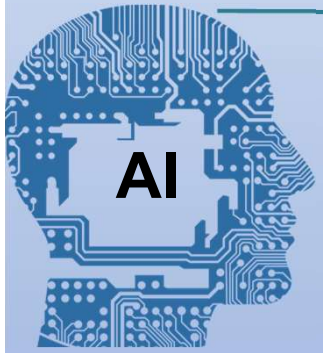


Utilizing clinical samples as a basis for the discovery of new drugs.

## Targeting disease signature instead of a single target[1]

Analyzing multi-omics integrated signatures from hundreds of patients at the cellular and tissue level. We *generate a more complete picture of the disease-relevant processes.*

## *An innovative AI-based pipeline*




Building a strong basis for defining new drugs having the potential to reverse disease progression through computational methods.

## Validation in preclinical models

Demonstrating benefit of the candidate drugs in relevant preclinical model systems.

**Demonstrating patients benefit in appropriate clinical trials.**

**Application in patients to effectively treat the disease.**

# Our specific aim

To improve care for BC patients increasing their life expectancy and quality of life as a result of the novel drugs we introduce.

***New therapy for metastatic BC*** → ***Execution of a clinical trial phase I/ II*** → ***Life saving medicine***

**Exemestane, Fluvoxamine, Amiodarone**
(approved drugs)

*patients with terminal stage BC*

## The next step towards implementation is the execution of a clinical trial phase I/II

We have the full support from the patient organizations (like World Bladder Cancer Patient Coalition) and the relevant professional members (urologists and oncologists) from the European societies of Urology.

# Prediction of drugs with reversal potential of MIBC

1

870 proteins from the MOS proteomics database

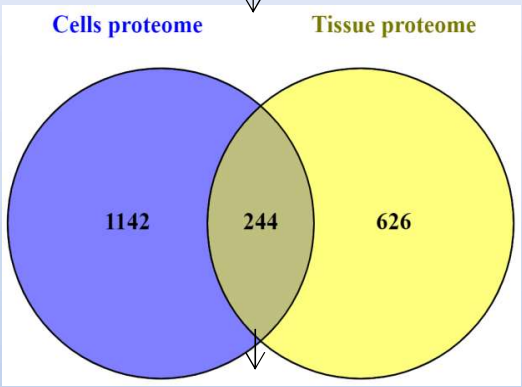
511 genes from the tissue transcriptomics data

427 features from the tissue data from the literature

1575 features with different trend of expression between MIBC and NMIBC tissues

2

Comparison of BC cell line proteomics with tissue proteomics using the MOS proteomics database




225 proteins common in cell lines and tissues with different trend of expression between MIBC and NMIBC

3

Comparison of BC cell lines transcriptomics and proteomics data

399 features common in BC cell line transcriptomics and proteomics data being significant in at least one dataset

399 features with different trend of expression between MIBC and NMIBC in BC cell lines

ConnectivityMap

ConnectivityMap

ConnectivityMap

**23 drugs/compounds**

with consistent negative enrichment score (threshold <-0.5) with reversal potential of MIBC

*Data produced in Mosaiques*

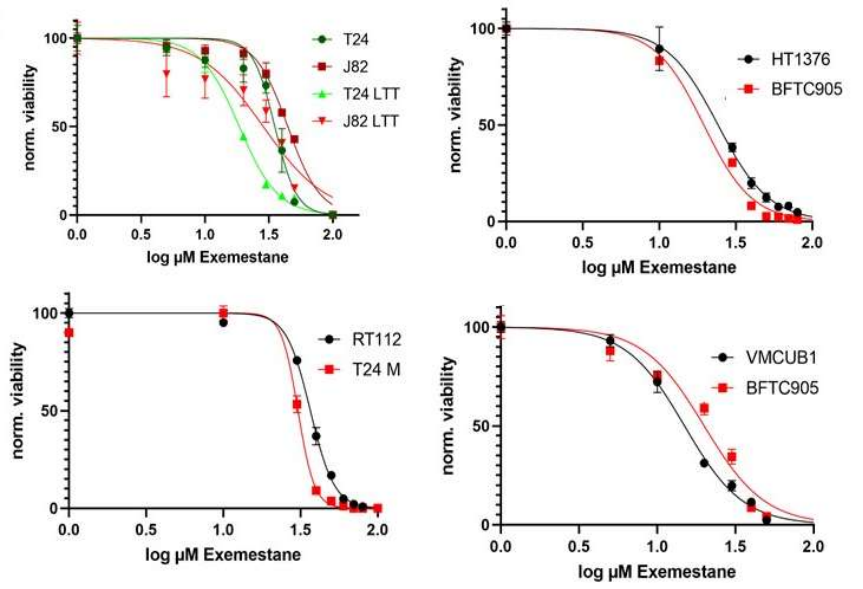
# Candidate drugs for *in vitro* study

✓ **13 compounds (out of the 23) were prioritized for *in vitro* screening based on their novelty in the context of BC and their availability**

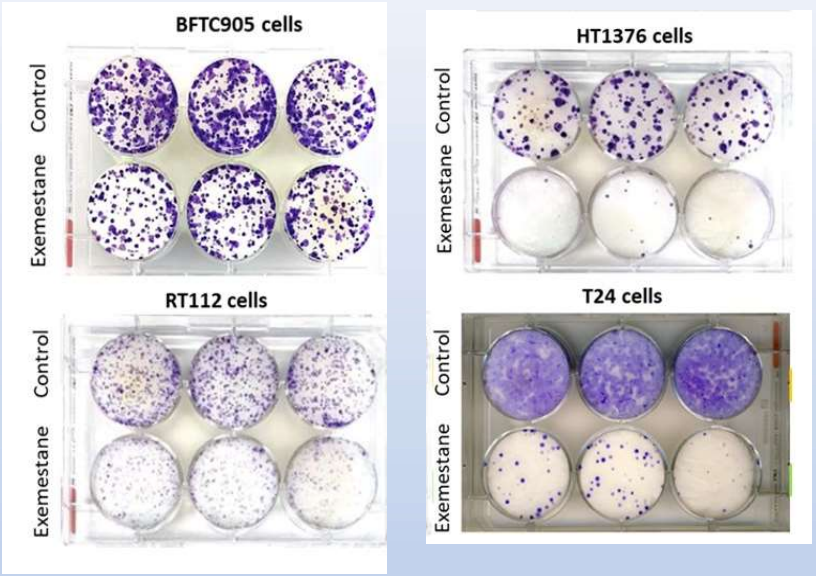
| Name | Class | *In vitro* impact at low concentrations |
|---|---|---|
| ursodeoxycholic acid | secondary bile acid | |
| **cephaeline** | **alkaloid with emetic properties** | ☑ |
| sulfamethoxypyridazine | a sulfonamide antibacterial | |
| guanethidine | antihypertensive | |
| phenanthridinone | PARP inhibitor | |
| **exemestane** | **antiestrogens** | ☑ |
| DL-thiorphan | enkephalinase inhibitor | |
| trazodone | antidepressant<br>serotonin receptor antagonists and reuptake inhibitors | |
| N-phenylanthranilic acid | non-steroidal anti-inflammatory drug | |
| oxamic acid | competitive inhibitor of pyruvate | |
| **1,4-chrysenequinone** | **activator of aryl hydrocarbon receptor (AhR)** | ☑ |
| fenspiride | Antiasthmatic & COPD Preparations | |
| morantel | anthelmintic drug | |

# Exemestane

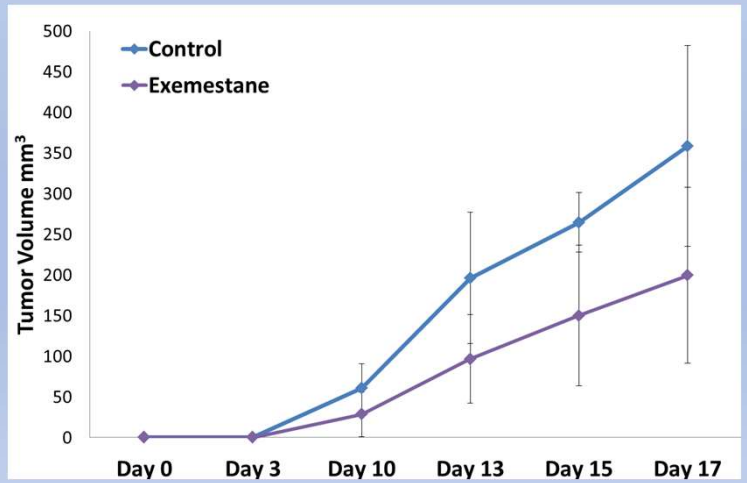
## Decreases proliferation in vitro



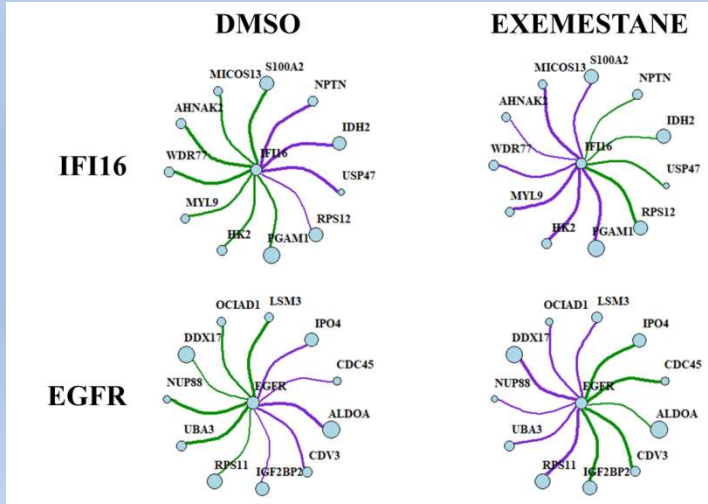

## Decreases colony formation capacity




## Reduces BC tumor growth *in vivo*

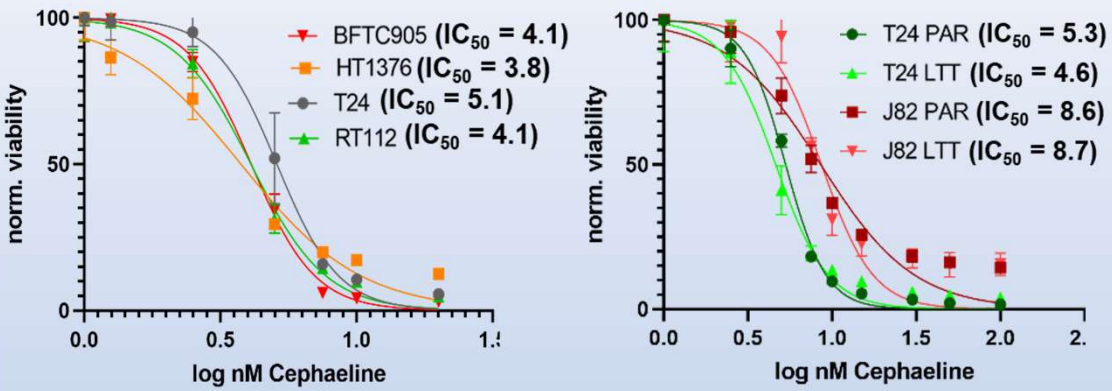



## *Targets the EGFR and IFI16 networks*



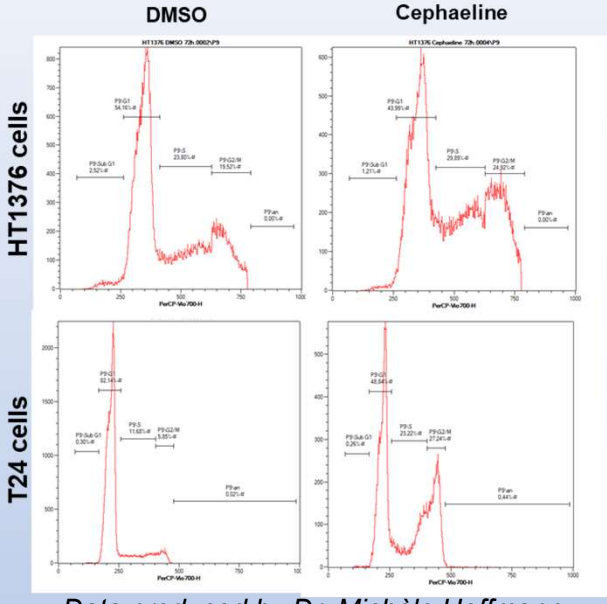
# Cephaeline

## Decreases proliferation in vitro



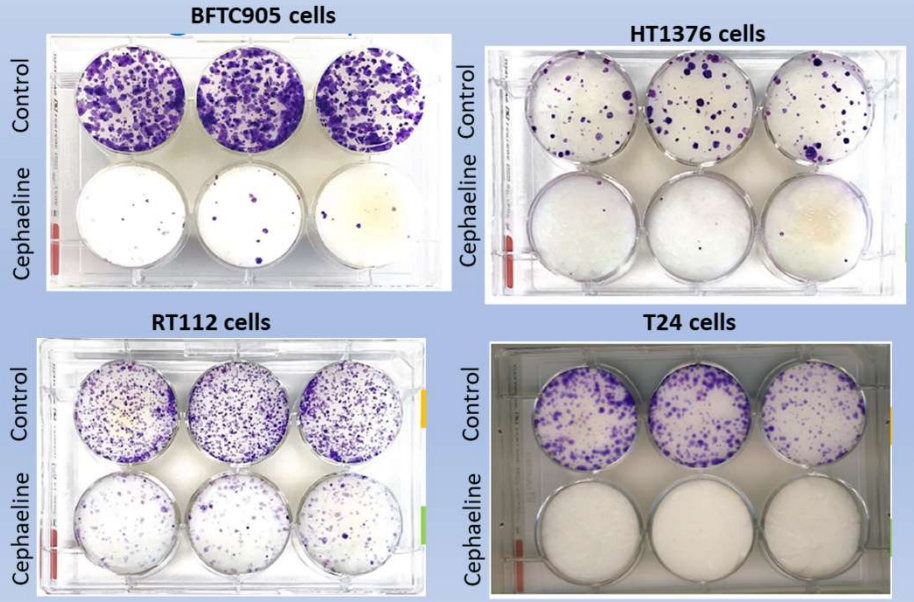

*Data produced by Dr. Michèle Hoffmann*

## Induces cell cycle arrest



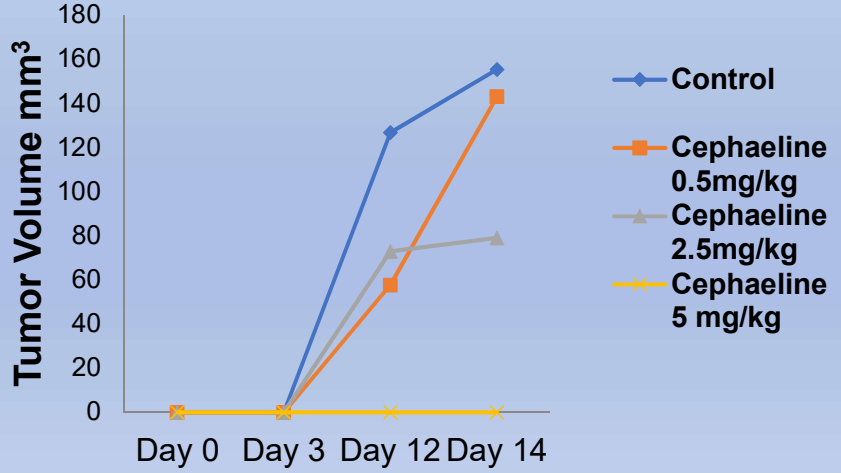

*Data produced by Dr. Michèle Hoffmann*

## Decreases colony formation capacity




*Data produced by Dr. Michèle Hoffmann*

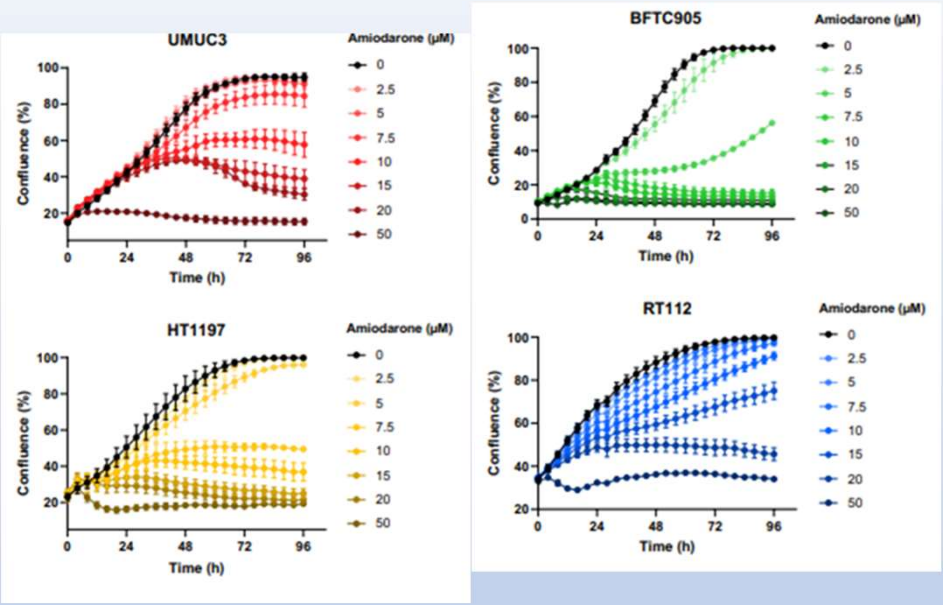
## In vivo impact but with high toxicity



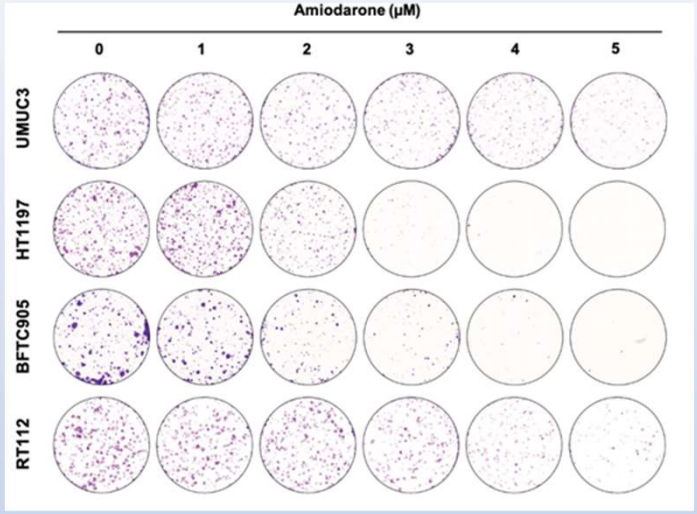

*Data produced by Dr. Maria Roubelakis and Dr. Antonia Vlahou*

# Amiodarone

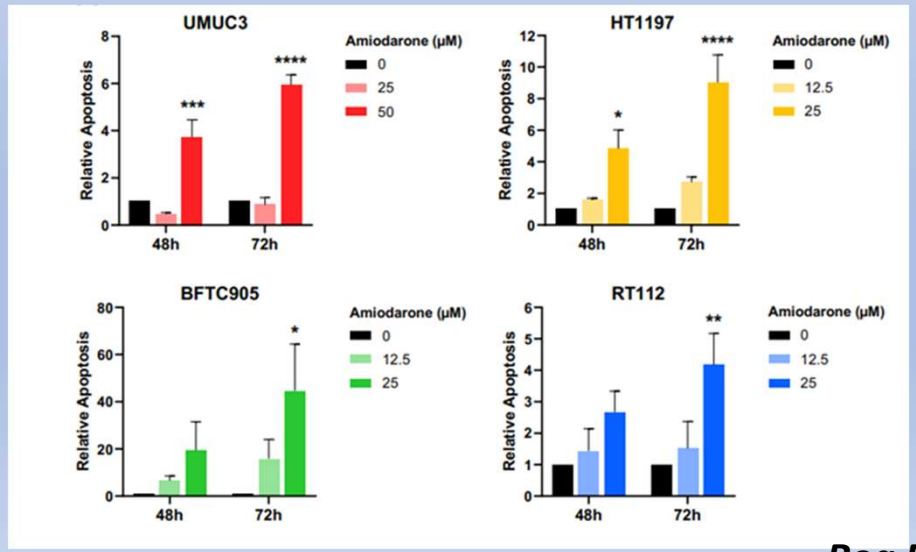
## Decreases proliferation in vitro



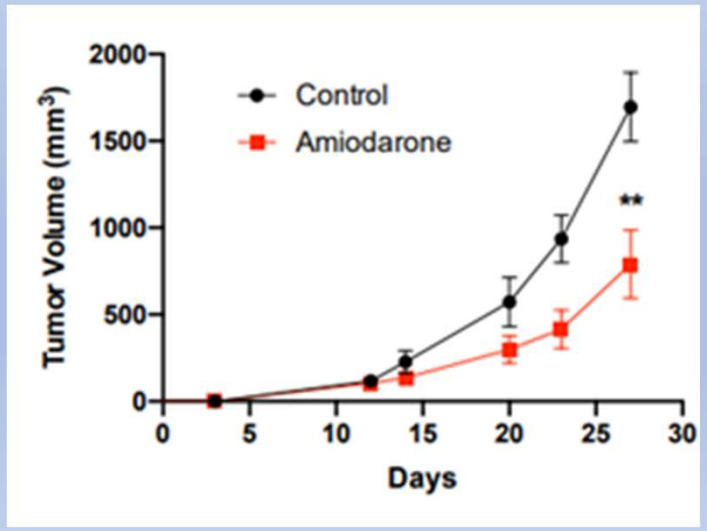

## Decreases colony formation capacity




## Induces apoptosis in BC cells




## Reduces BC tumor growth *in vivo*




***Roa F. et al.*** **Cancer Research Communications (revisions)**

# Summary – The Roadmap

| Drug/ compound | Project | FDA approved | In vitro impact confirmed | In vivo impact confirmed | Stage |
|---|---|---|---|---|---|
| Exemestane | ReDrugBC | YES | ☑ | ☑ | Ready for clinical trials |
| Amiodarone | REDIRECT | YES | ☑ | ☑ | |
| Fluvoxamine | REDIRECT | YES | ☑ | ☑ | |
| Cephaeline | ReDrugBC | NO | ☑ | ongoing | Preclinical |

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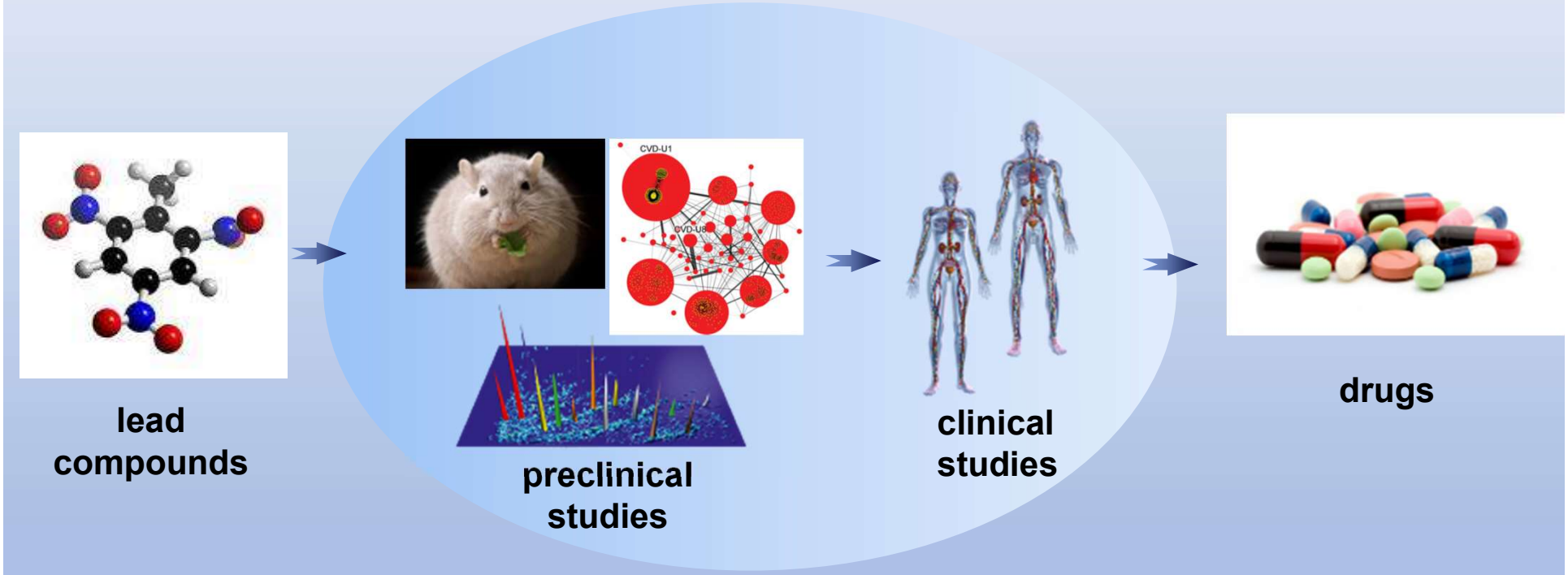
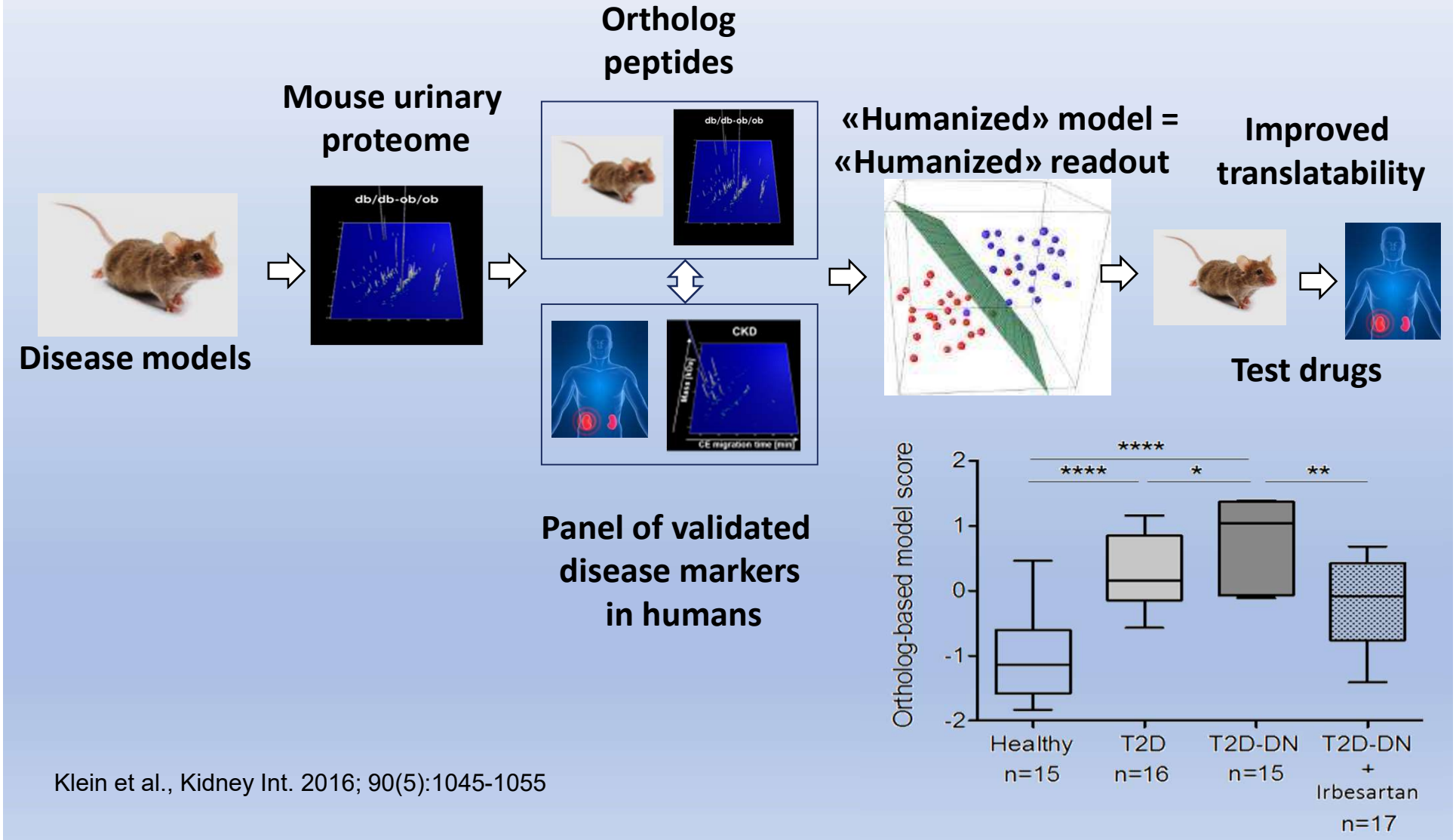
# Proteomics facilitates drug development




Choosing the appropriate animal model will ease the transition from preclinical to clinical studies and improve the success of the clinical trial.

# »Humanized» model concept




Klein et al., Kidney Int. 2016; 90(5):1045-1055

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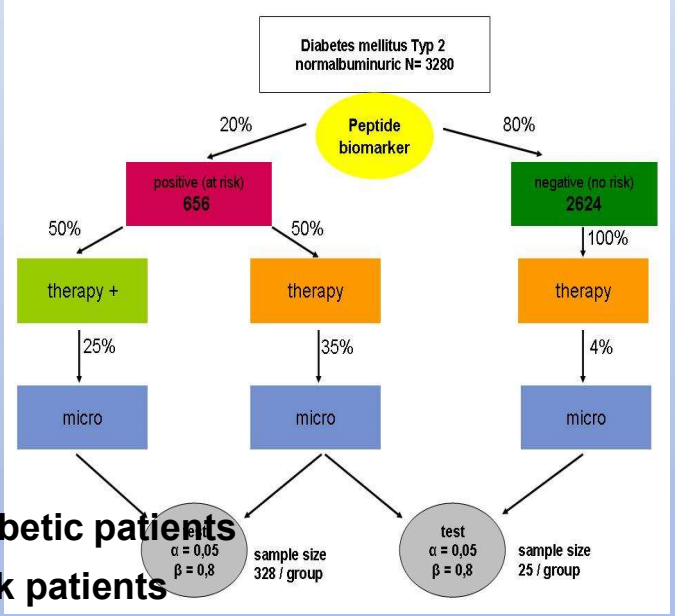
# CKD273 improves patient stratification

EU PRIORITY

Early prediction of diabetic nephropathy
through urinary proteome analysis

- Multicenter study
- 15 partners in Europe
- 6 years

## RCT employing CKD273 for stratification
## Targeted therapy/personalized medicine in Nephrology




- **1770 normoalbuminuric type 2 diabetic patients**
- **Stratification into low and high risk patients**
- **High risk patients were randomly assigned to aldosterone blocker spironolactone 25 mg or placebo therapy on top of optimal standard therapy**

Lindhardt et al., BMJ open 2016, Tofte et al., Lancet D&E, 2020

# Benefit of CKD273 in Patient Stratification

**Scenario 1**
**without proteome analysis**

- Probability to reach the endpoint*: **7%**
- Power calculations for demonstration of 30 **% benefit of the drug** (decrease in reaching disease endpoint)
- Required number of patients to be enrolled: **n= 1992**

**Scenario 2**
**with proteome analysis**

- **Pre-selected Patients**, Probability to reach the endpoint*: **20 %**
- Power calculations for demonstration of 30 **% benefit of the drug**
- Required number of patients to be enrolled: **n= 616**

**Huge reduction of costs!**

***transition from CKD stage 2 to 3**

# Areas of application - Available tools

| | Kidney diseases | Prostate Cancer | Bladder Cancer | Heart Failure | Coronary Artery DIsease |
|---|---|---|---|---|---|
| Urinary proteomics | ✓ | ✓ | ✓ | ✓ | ✓ |
| Tissue proteomics | | ✓ | ✓ | ✓ | ✓ |
| Animal models | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ongoing Clinical Trials | ✓ | | | | |

# EU collaborative projects

## Oncology

Chromatin3D

BCMolMed

TransBioBC
Translation of novel Biomarkers for Bladder Cancer for clinical outcome prediction

TransPot

## Cardiovascular disease

HOMAGE

PRETREAT

SysVASC

EU-MASCARA

## Kidney diseases

iMode-CKD

EURenOmics

European Commission
RENALTRACT

SysKid

EU PRIORITY

TranCYST

# Status quo

- Areas of interest: kidney diseases, prostate and bladder cancer, heart failure and coronary artery disease.
- Networks with leading clinicians, bioinformatics, animal experiments experts, etc. established.
- Human proteome data sets (tissue and urine) are determined, data evaluated, diagnostic classifiers established and tested, bioinformatics evaluation runs, first drugs defined.
- The data sets of animal models are established and the homology to humans is investigated to define best suited animal models.
- Preclinical studies in animal model (for bladder cancer) successfully completed.
- First large, multi-center RCT completed (for chronic kidney disease).

# International Network of R&D Partners