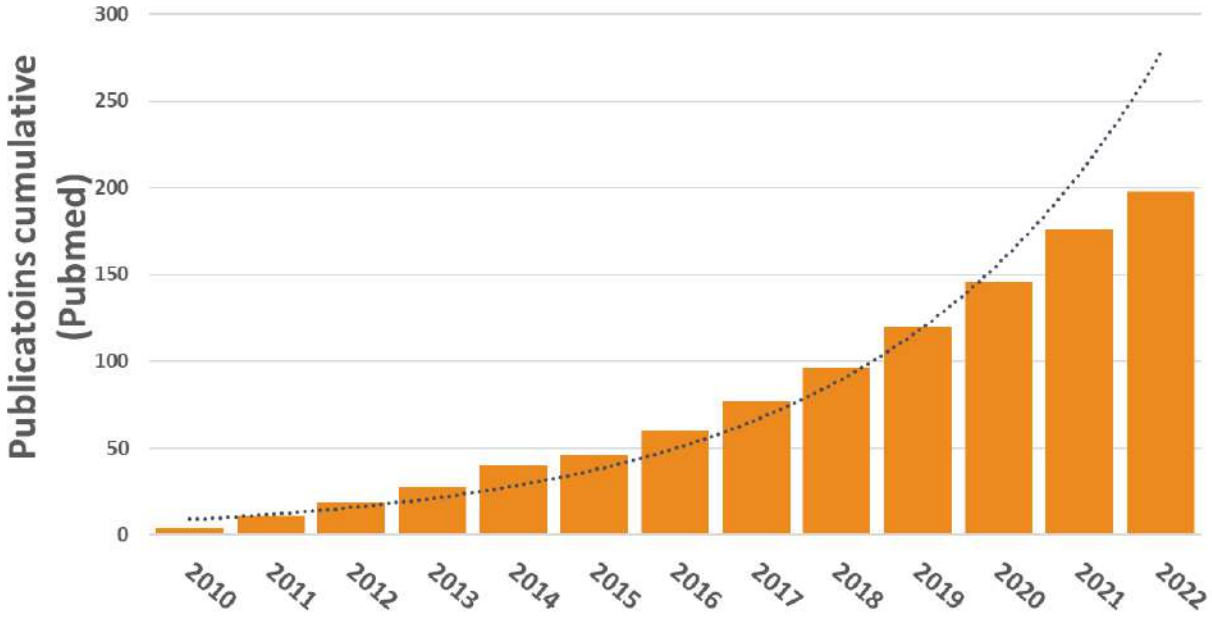
# Introducing an unbiased approach that uses Multiplex Kinome Activity Profiling

Tracking changes in cellular signaling and pathways with PamGene

PamGene

# Pamgene overview

- Spin-off from the Organon Teknika
- Contract Research Services and platform technology
- Specialized in signaling protein biomarker technology
- Extensive body of peer reviewed publications on technology
- Patented multiplex peptide biomarker profiling technology (15 patents)

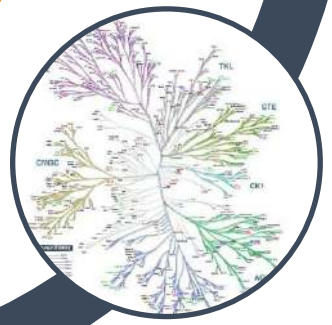




# Trial overview

**Direct access to our approach during this trial period of typically 12 months**

**Multiple groups invest together and collaborate using the approach and incorporate us in new funded projects**

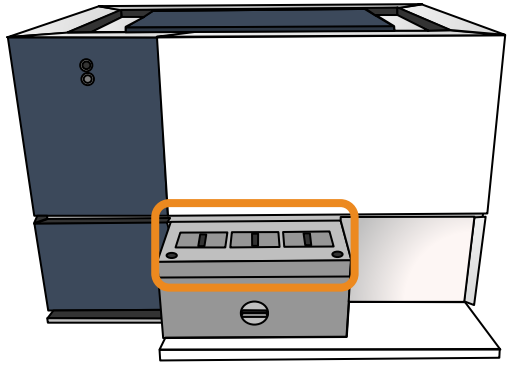


**The investment of consumables is including our direct scientific support.**

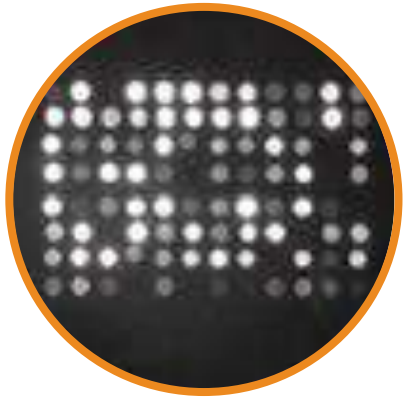
**E.g.: Experimental design, data analysis, reporting, interpretation**

# Technology summary

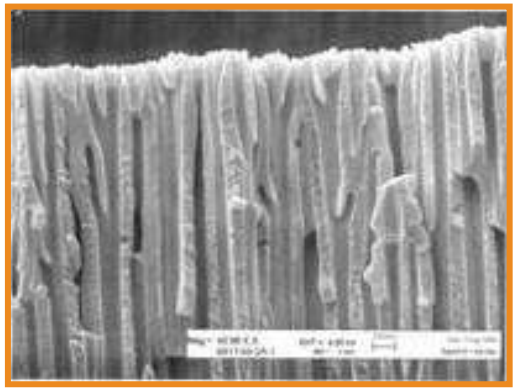
## PamStation 12®



## Pamchip 4®



## 3D membrane



### Reader instrument

- Capable of loading 3 Pamchips per experiment, containing 12 samples
- High throughput, only 15 min's hands on time

### Consumables

- Serine /Threonine Pamchip (STK) with 144 targets / substrates
- Tyrosine Pamchip (PTK) with 196 targets / substrates

### One of 4 Arrays

- All arrays are identical
- Generic antibody binding to all phosphorylated targets / substrates

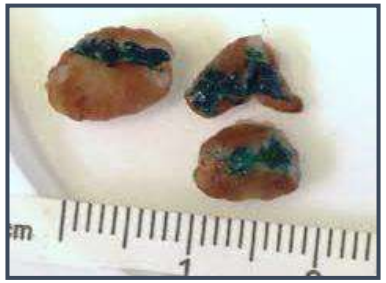
### Porous structure

- Peptides are spotted in high concentrations, making the assay sensitive.
- During incubation, the lysate is pumped back and forth through the membrane, this makes the assay fast.

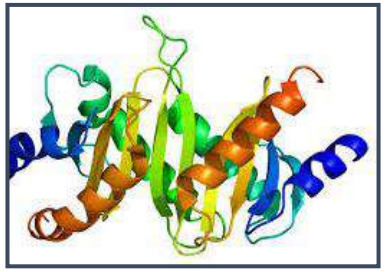
# Input requirements

**0,5 – 5,0 µg** total protein per array
**10.000 – 50.000** cells per array

**Primary and cultured cells**
PBMCs, WBC, platelets, Bone marrow
Primary cells
Culture cells (adherent or suspension)



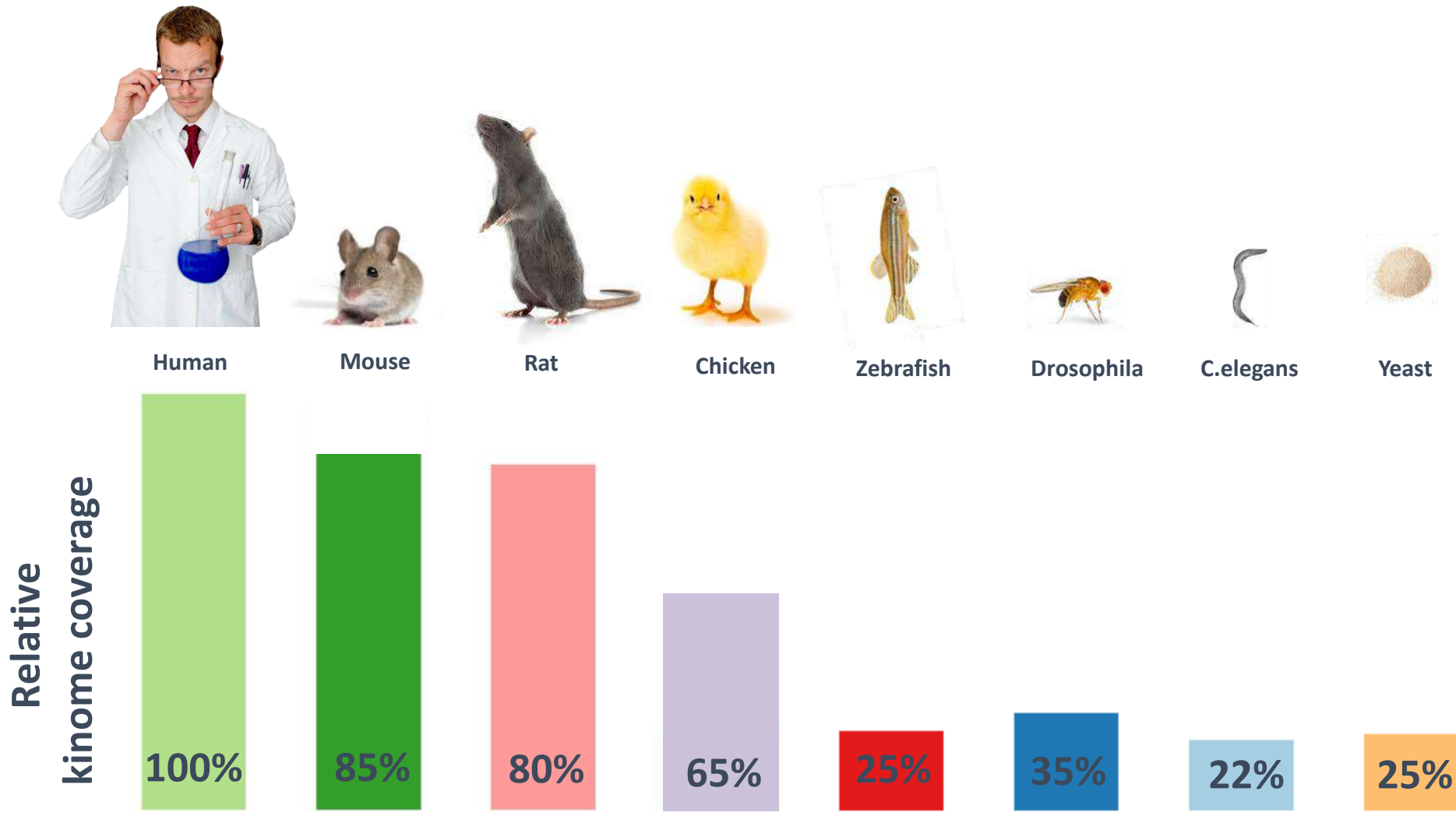
**Primary biopsies, slices, clinical samples**
Freshly frozen (alternatively Tissue-Tek)
Tumor content advise >70%
Different tissues (FNA) e.g. Colon, lung, liver, breast, brain, prostate, skin, thyroid, CSF



**Purified proteins**
Recombinant kinases

**Collect samples over time, fresh freeze, and store for long periods at -80°C**

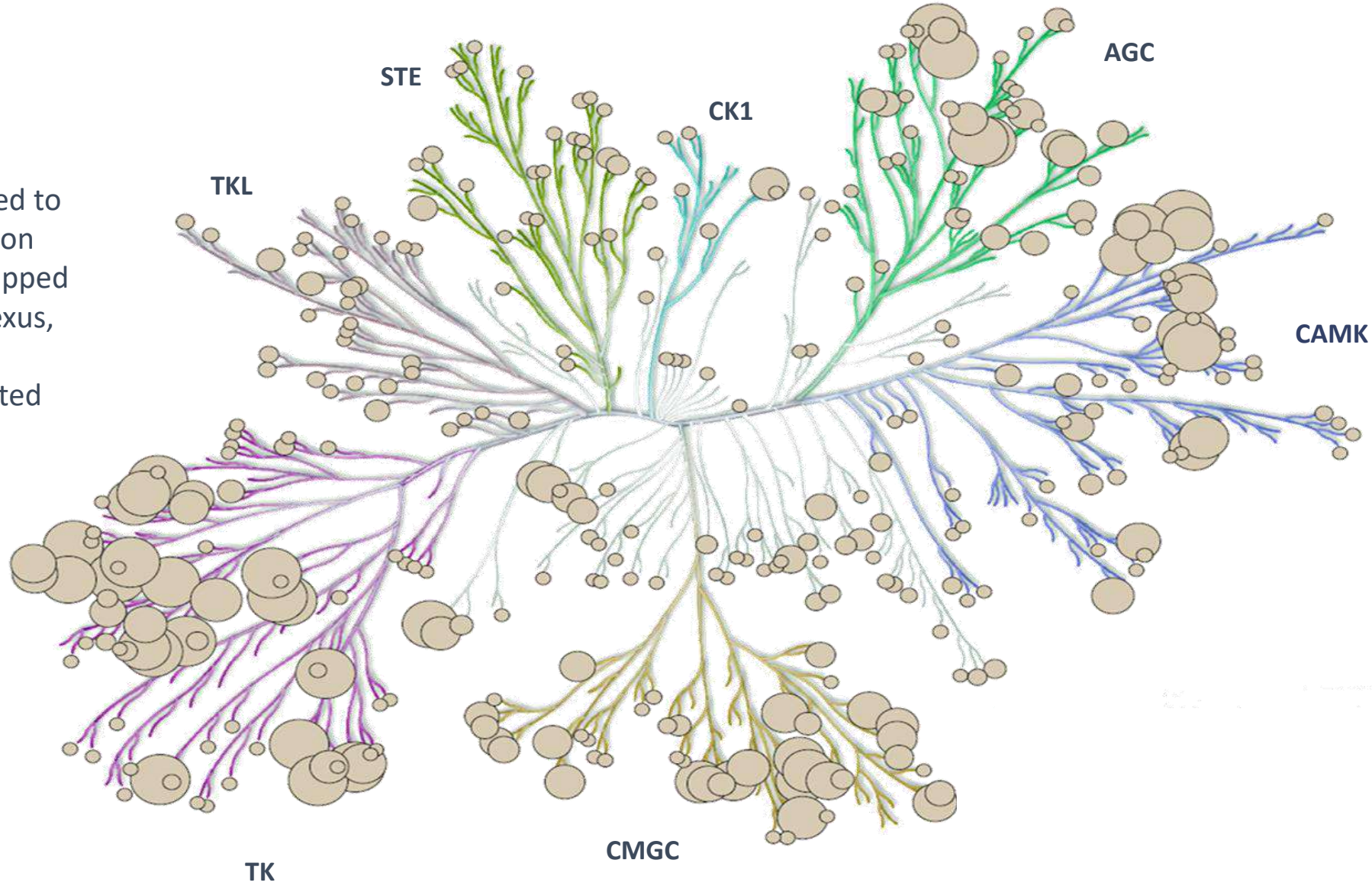
# Organism coverage



# Kinome Coverage

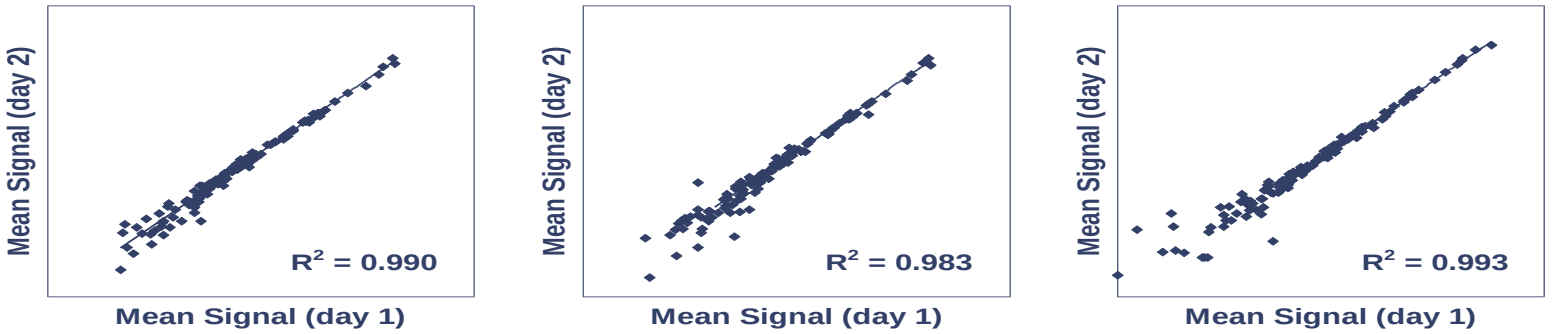
## Covering >380 Kinases

Kinases that are known or predicted to phosphorylate substrate peptides on the PTK and STK PamChip® are mapped from select databases (HPRD, Kinexus, Phosphosite PLUS, Reactome, Phopho.elm, UniPROT) and projected on the kinome tree.

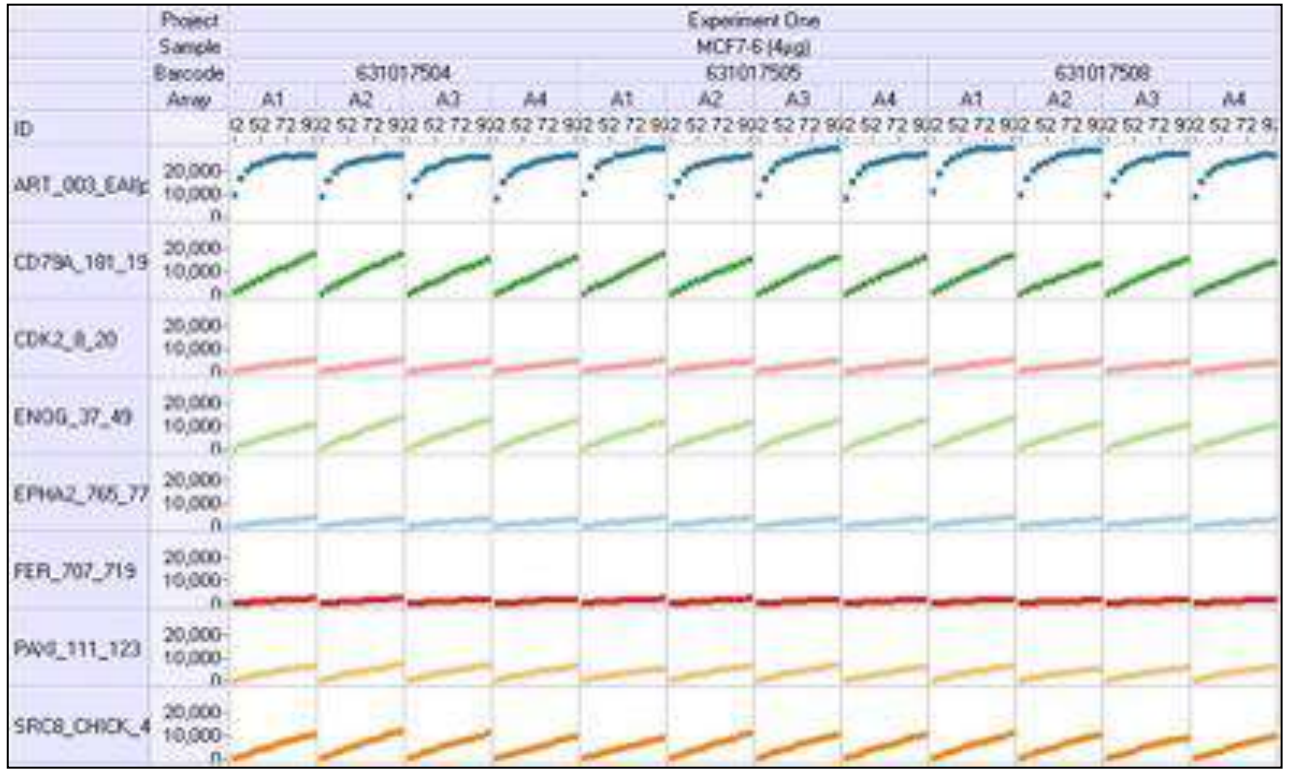


# Reproducibility

Day-to-day reproducibility




Technical reproducibility within a single run with a variance 5-10%.



# Applications

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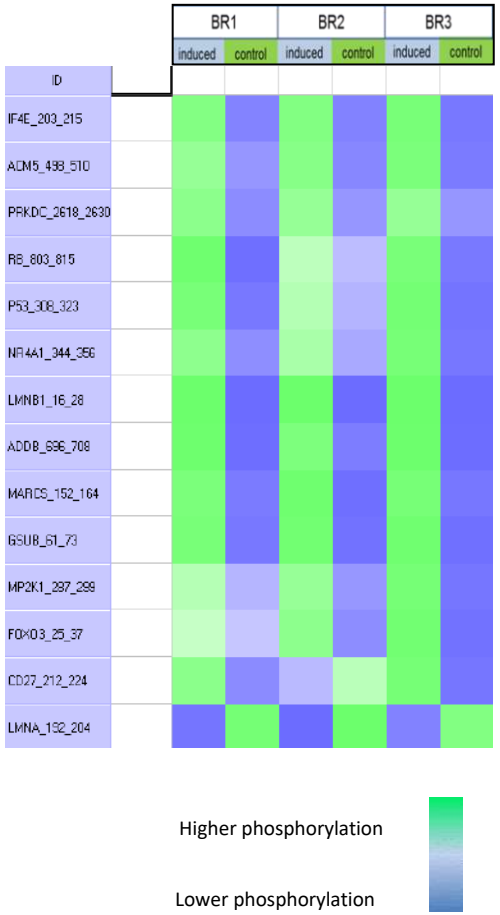
| Fundamental and Discovery research | Biomarker and Clinical research |
| --- | --- |
| • Signaling & Pathway elucidation | • Therapy-predictive biomarkers |
| • Target discovery | • Prognostic biomarkers |
| • Target interaction | • Classification biomarkers |
| • Disease model characterization | |

# Pamchip reveals increased MAPK signaling signaling

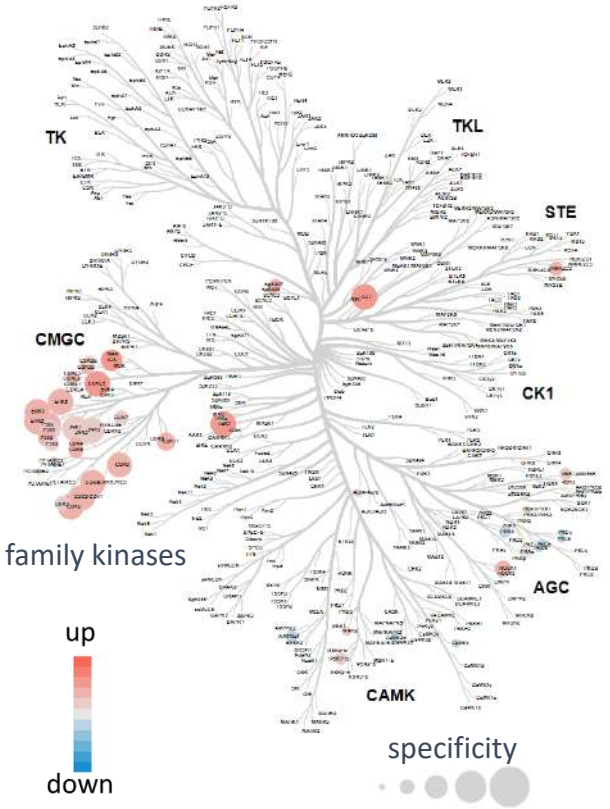
## Overexpression of Raf-1 is expected to result in an increase of ERK (MAPK) signaling

Significantly different phosphosites between induce en control

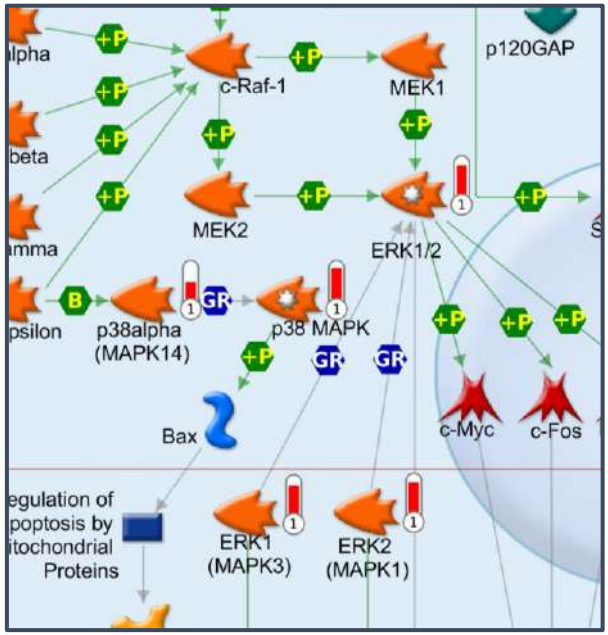



Upstream kinase analysis, based on Kinase to substrate interaction recorded in know databases


Upstream kinase analysis visualized on a kinome phylogenetic tree and Metacore pathway analysis




MAPK family kinases

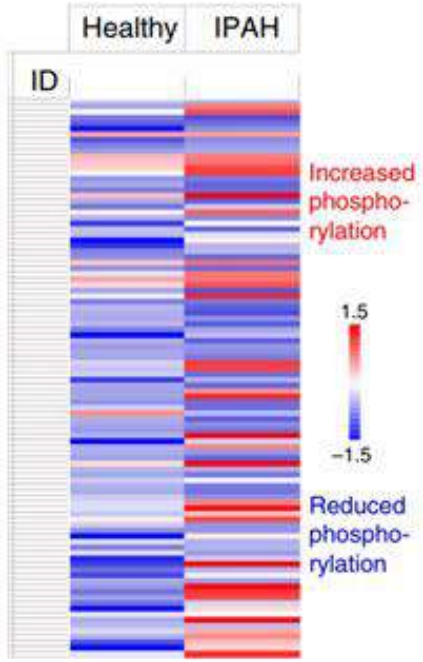


# Pamchip reveals increased activity of the CDK signaling pathway

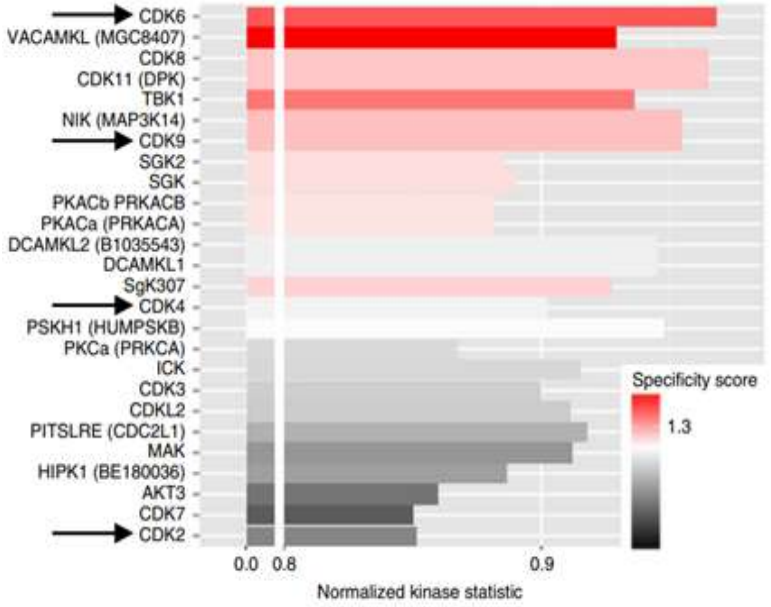
## Profiling of primary pulmonary cells from healthy and IPAH patients

### Validation

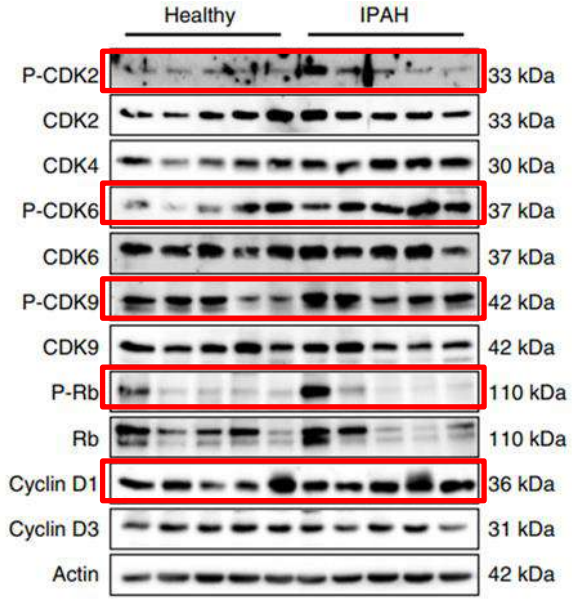
Differential peptide analysis



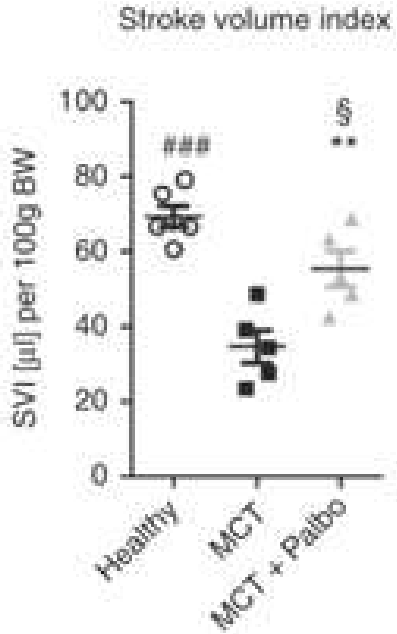

Upstream Kinase Analysis




Western blot




*In vivo* proof
MCT rat Model




IPAH: Idiopathic pulmonary arterial hypertension

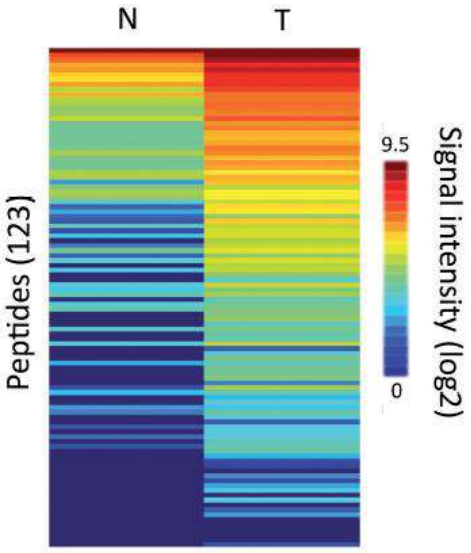
MCT = Disease inducer
Palbo.= CDK inhibitor


Weiss *et al*.: Nature Communications 2019

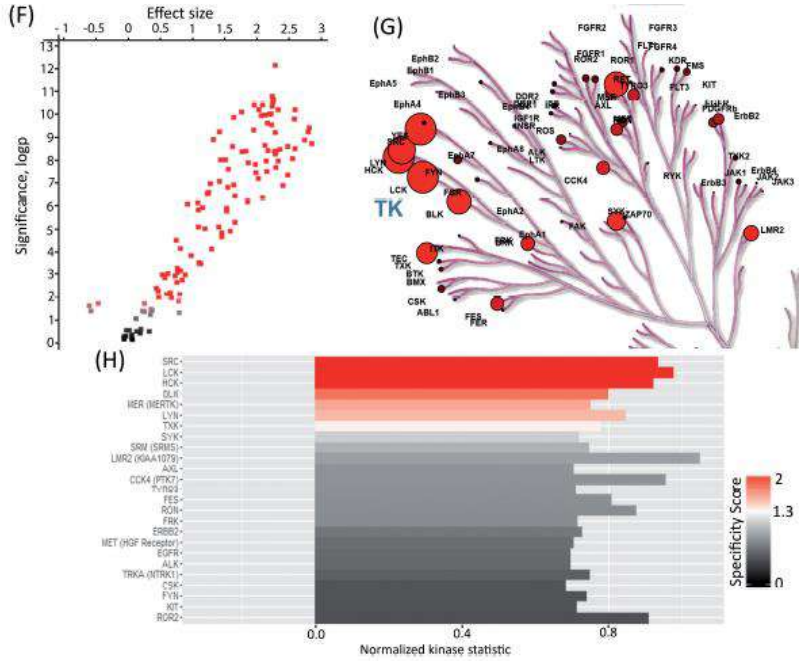
# Increased activity of Src family signaling in HNSCC tumours

## Profiling of tumour vs healthy tissues in HNSCC patients

Differential peptide analysis



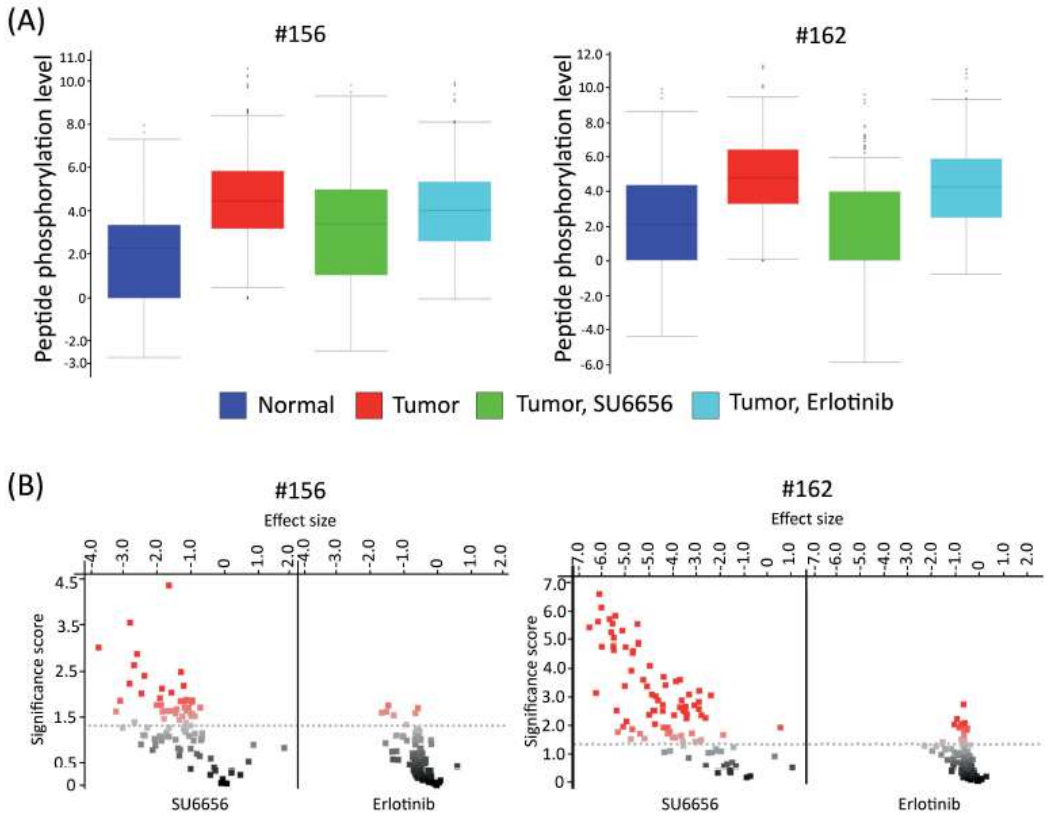

PamGene PTK Analysis




*HNSCC, head and neck squamous cell carcinoma*

## Validation

*Ex vivo* inhibition of patient lysates with SFK inhibitor SU6656

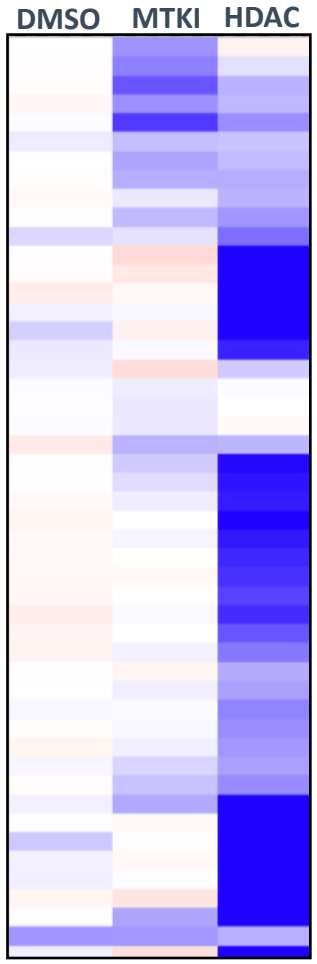


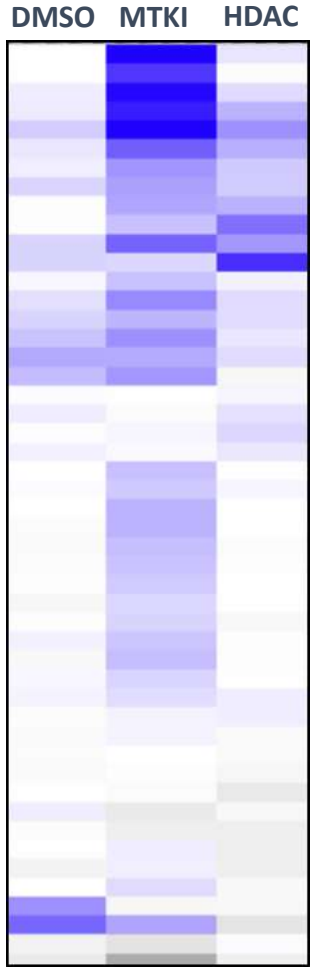
# Directly adding kinase inhibitors in lysate

PamGene

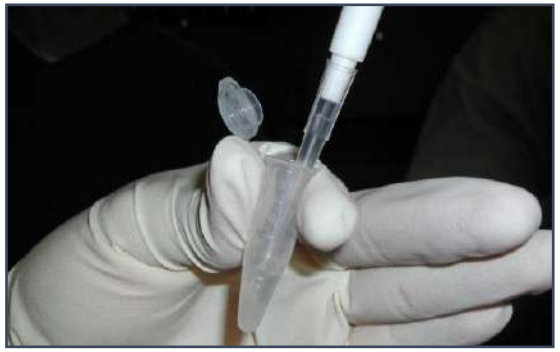
Cellular Treatment

Also non-kinase inhibitors (HDAC inhibitors) can be profiled on their indirect effect on kinases in cells.




Lysate Treatment

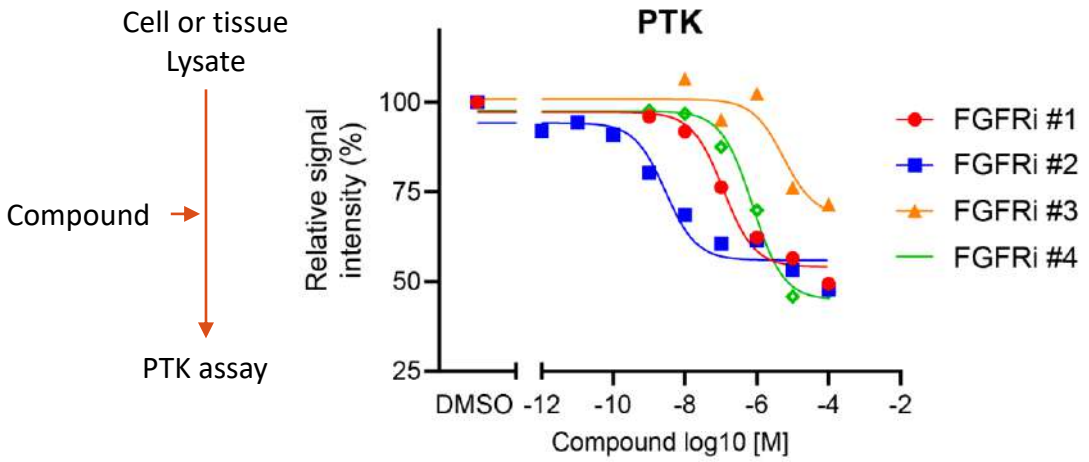


4-fold inhib.

No inhib.

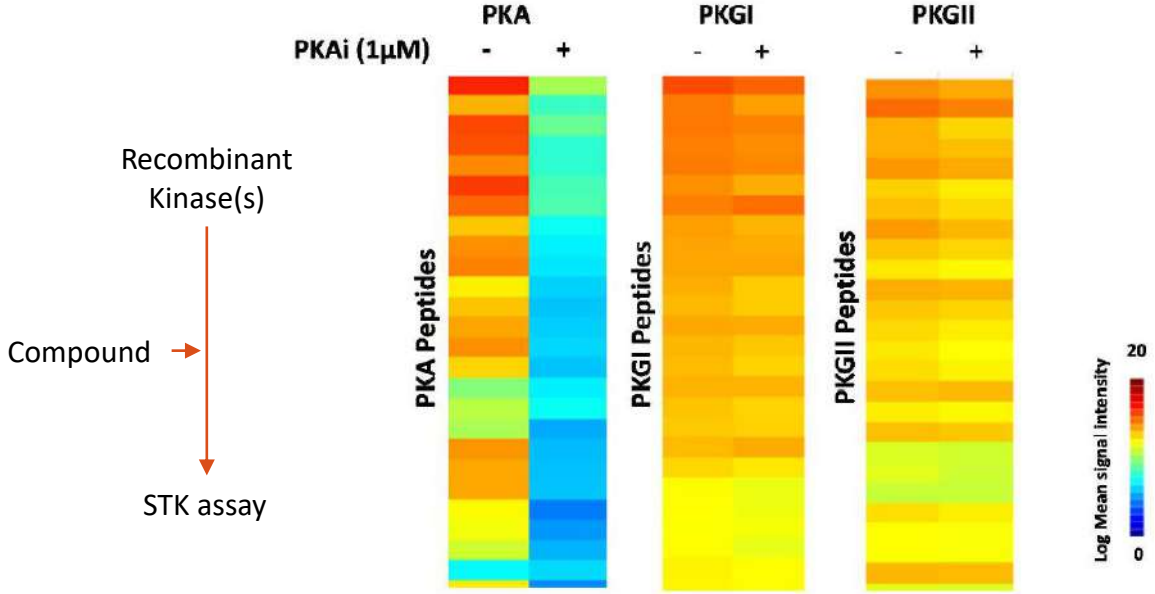
# Spike-in and recombinant experiments

## *In vitro* $IC_{50}$ determination of novel FGFR inhibitors in specific cell lysates




Courtesy of Services

## Specificity of PKA inhibitor on recombinant PKA and PKG




Roy *et al., Int. J. Mol. Sci.*, 2021

# Consumable investment


**A single Trial Kit Purchase of € 4.000,00 euro.**

- **Each kit contains a single type of 12 PamChips, separately sealed.**
  **That translates to In total 48 identical arrays (STK or PTK), to investigate 48 samples**
  **Necessary reagents are included**

- **This investment includes:**
  **The installation of the instrument on location;**
  **Training of users in instrument handling & wet lab;**
  **We perform data analysis, shared in personalised reports;**
  **Scientific support for preparation and interpretation.**

- **To allow us to start, we need enough interest on a specific location, typically that translates to 5 - 8 groups that invest 10-16 kits for their research purposes.**

**Reach out at: jlebens@pamgene.com**

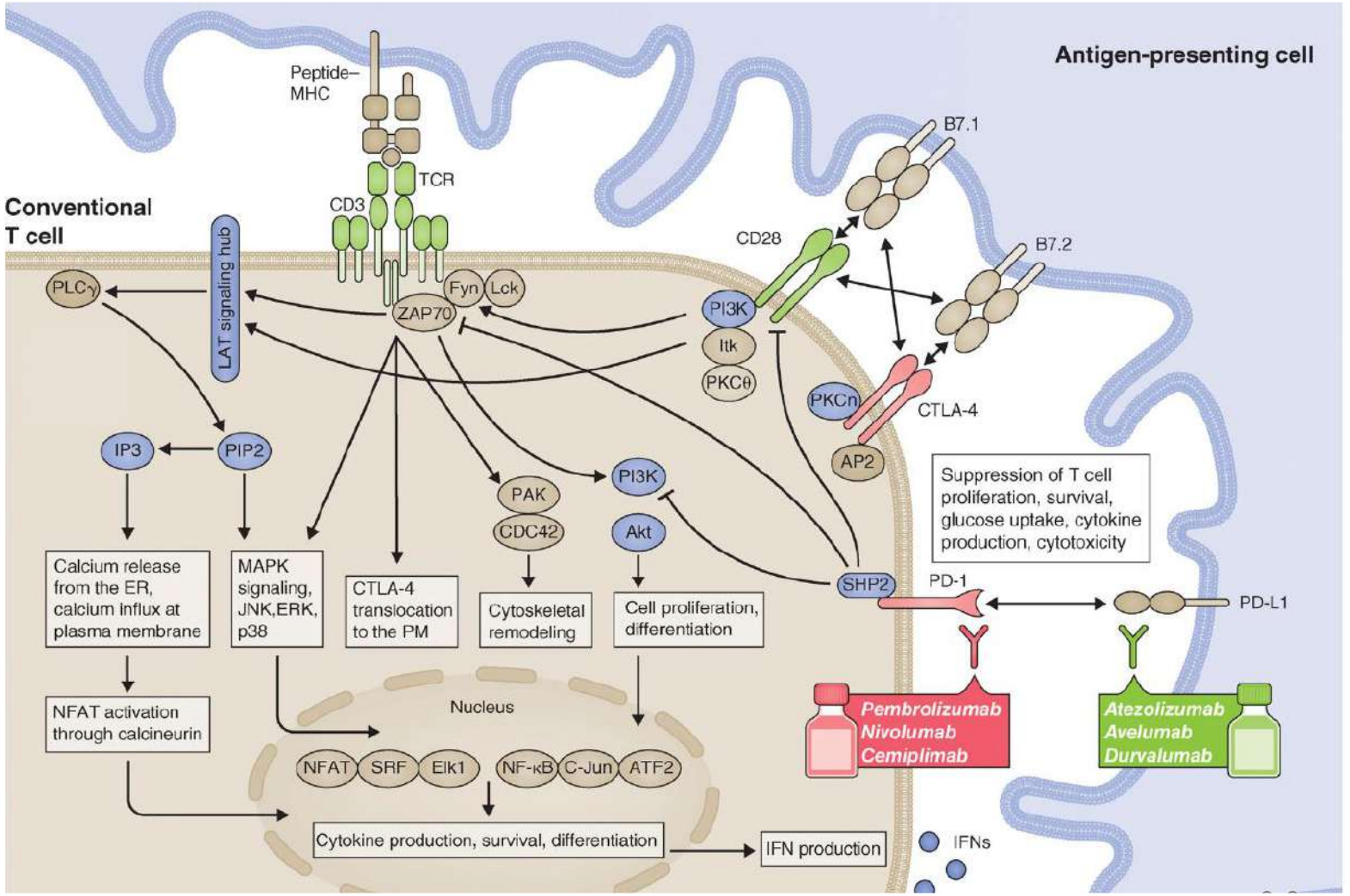
# Pamgene IVD certification June 2022

A quick Introduction to our internal IVD development program

# Diagnostic Application

Kinase activity impacts how individual patients respond to PD-1 and CTLA-4-directed therapies.

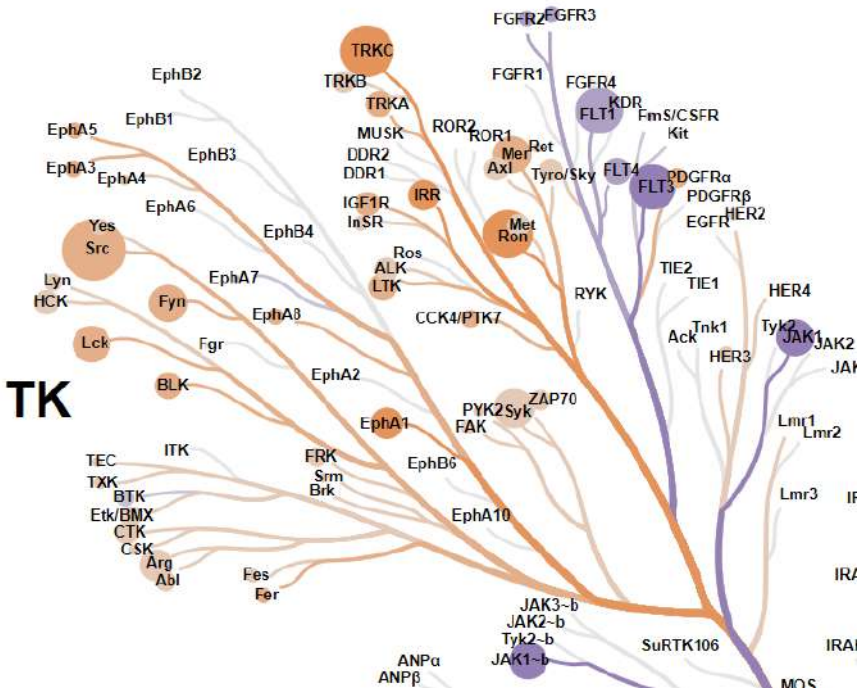
Our diagnostic response prediction is done by profiling whole blood PBMC samples, taken from NSCLC & Melanoma patients prior to treatment.



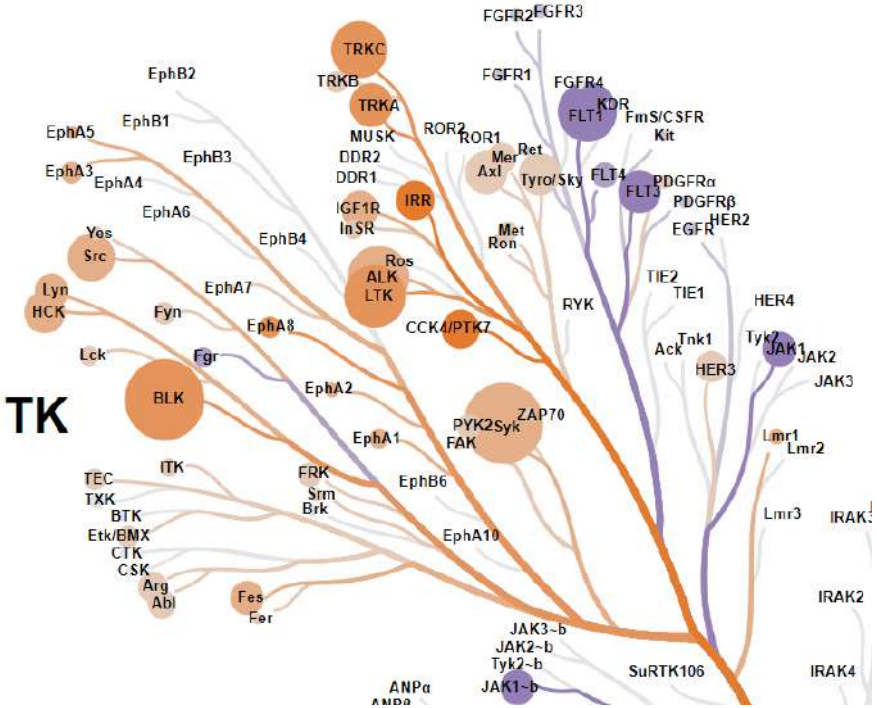
# Kinase Fingerprint

## Differential kinases between responders and non-responders

Melanoma Cohort
PD20 / Clinical calibration




NSCLC Cohort
PD12 / clinical calibration



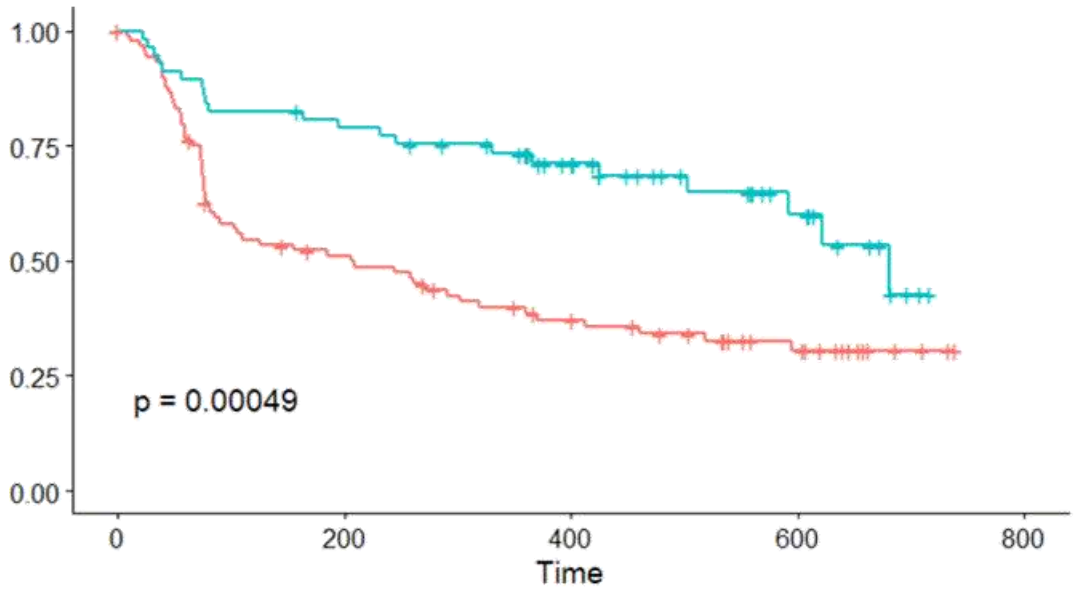

Responder

Non-responder

# Prediction performance in Melanoma valdiation study

- Study design: 160 patients with advanced melanoma and eligible for ICI treatment (Standard of care), multi-center study.
- Prospective trial, 5 clinical centers (calibration & validation)
- Two cohorts (160 samples )have been analysed:
  - Centralized, standardized PMBC isolation.
  - Samples analysed in Diagnostic Assay Services Facility.
  - Kinome multiplex array data analysed
- Patient response is assessed by
  DR (durable response ) and PFS (Progression Free Survival).
- Accuracy in independent validation cohort is **70-76 %** for immune treatments
- Hazard Ration(HR) for the survival analysis ranges from 1.4 to 2.1
- Multimodal analysis ongoing (clinical chemistry, hematology)
- **IO**pener® test is predictive for disease progression in Melanoma patients treated with anti-PD1. (first line and/or second line immune treatment)

**Melanoma validation studies**
Combined cohorts -> DLM model

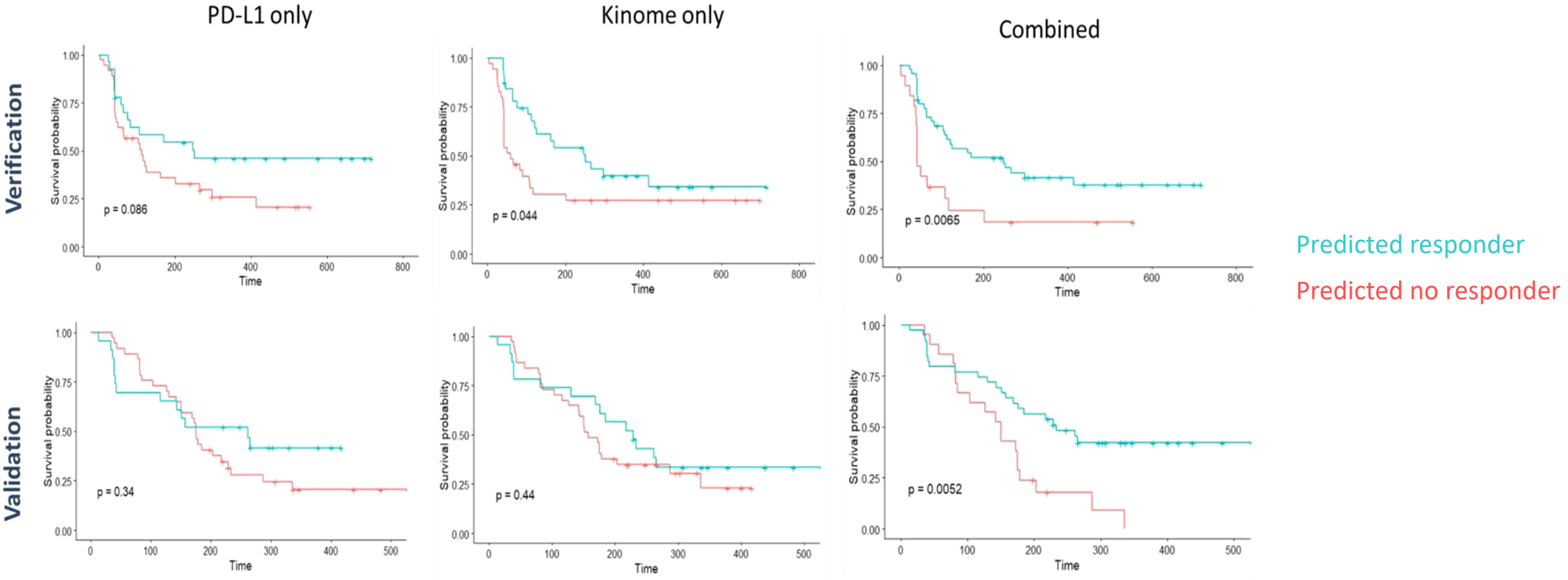



Predicted responder

Predicted no responder

# Prediction performance in NSCLC valdiation study

- True and false positive/negative responders are assessed for PD-L1 and **IO**pener® prediction score in NSCLC validation studies (n=150)
- **IO**pener® in combination with PD-L1 test is superior predictor of the response of NSCLC patients treated with ICI therapy
- Hazard Ratio (HR) for TPS score is 1.3 and HR is 2.3 when combined with IOpener (Accuracy Is 72% for TPS<50%)
- **IO**pener® test as an inclusion criterion will significantly improve the response to combo-therapy (TPS < 50%) compared to mono therapy (TPS>50%).



# A view on our customers

PamGene

Pharmaceutical Companies
Biotechnology Companies
Academic Medical Centers
Research Institutes
Laboratory Services Providers

Johns Hopkins University
AMGEN
APTINYX
Université Fédérale Toulouse Midi-Pyrénées
Johnson & Johnson
University of Warsaw
NIH National Cancer Institute
MERCK
Galápagos
Roche
umcg
NOVARTIS
UKE Hamburg
University of Copenhagen
AstraZeneca
Bristol-Myers Squibb
WWU Münster
Takeda
Tata Memorial Centre
Université de Strasbourg
janssen
Netherlands Cancer Institute
Antoni van Leeuwenhoek
The University of Toledo
Hellenic Republic National and Kapodistrian University of Athens Est. 1837
gsk
Nanyang Technological University Singapore
University of Zurich[UZH]
The Francis Crick Institute
Ludwig Cancer Research
Stockholm University
Nestlé
dkfz. German Cancer Research Center in the Helmholtz Association
anagenesis Biotechnologies
VU medisch centrum
Université de Lille
CECAD Cologne
Radboud University Nijmegen
LUMC Leids Universitair Medisch Centrum
institut cochin Centre de Recherche
Cincinnati Children's
Erasmus MC University Medical Center Rotterdam
UKSH Universitätsklinikum Schleswig-Holstein