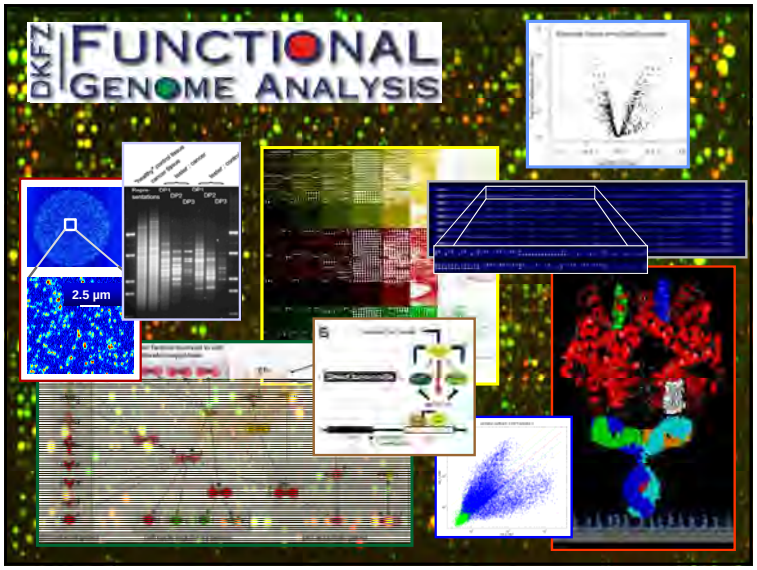
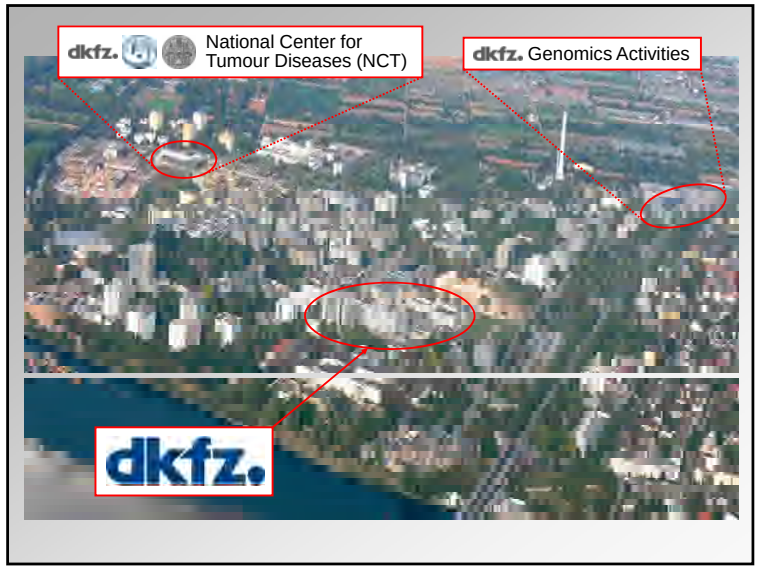
Name : Dr. Joerg Hoheisel
Born : In Bad Nanheim, Germany
Studies : At University of Konstanz, Germany
PhD : In 1984 – 1988
Postdoc : At Imperial Cancer Research Fund in London, UK 1988 – 1993

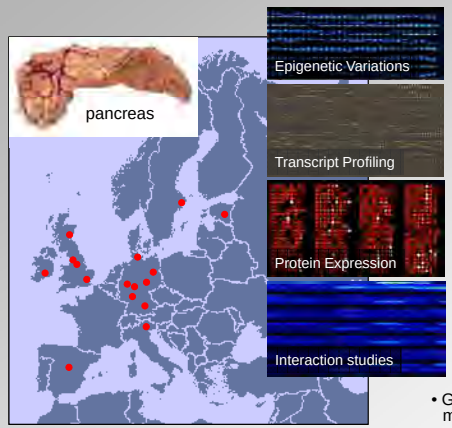
Since 1993 Group Leader at German Cancer Research Center (DKFZ)

Since 2008 Chairman of Scientific Council of DKFZ





Studying Pancreatic Cancer

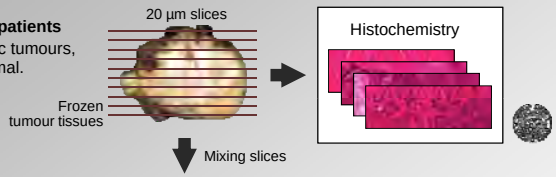


WP1: Epidemiology / ESPAC
WP2: Genetic profiles
WP3: Transcriptional profiling
WP4: Protein expression
WP5: Epigenetics
WP6: Molecular imaging
WP7: Prospective clinical trials

- Mouse models
- Kinase networks
- Protein interactions
- miRNAs as therapeutic targets
- Stroma activation
- Metastasis & local recurrence
- Protein secretion
- Genetically engineered mouse models for preclinical validation
- Therapeutic patient stratification

Studying Pancreatic Cancer

Material from 1200 patients
tumours T1-T4, cystic tumours, pancreatitis and normal.



Splitting in three homogenous fractions; separate isolation

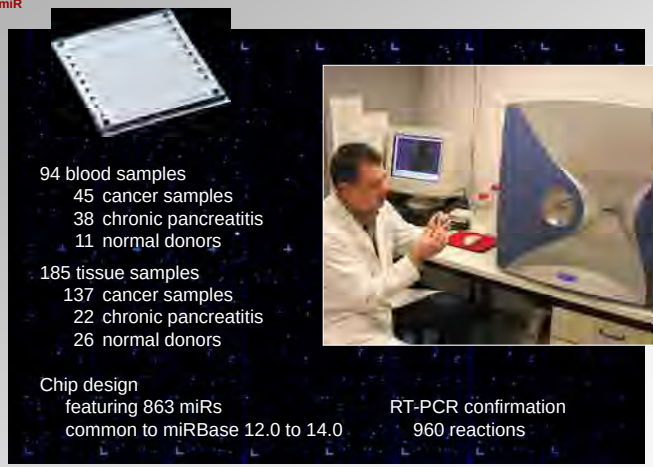
> 1000 DNA-samples	> 550 total RNA samples	> 700 protein samples
• Epigenetic analysis • Mutation analysis • SNP-typing	• DNA-microarrays > 550 mRNA analyses > 250 miR analyses	• Antibody-microarrays • Protein-microarrays

160 genomic sequences

> 350 blood & urine samples

MicroRNA (miR) Profiling

miR



94 blood samples
- 45 cancer samples
- 38 chronic pancreatitis
- 11 normal donors

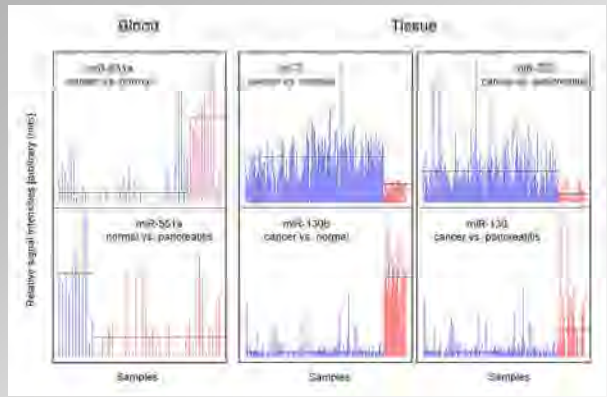
185 tissue samples
- 137 cancer samples
- 22 chronic pancreatitis
- 26 normal donors

Chip design
- featuring 863 miRs
- common to miRBase 12.0 to 14.0

RT-PCR confirmation
960 reactions

Individual miR Profiles

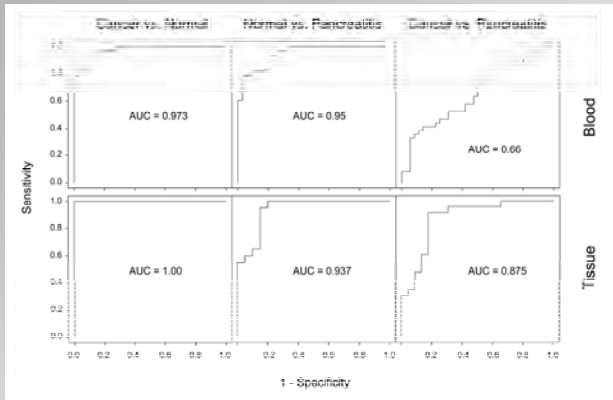
miR



Bauer *et al.* (2012) *PLoS ONE* **7**, e34151.

MicroRNA Diagnosis from Blood

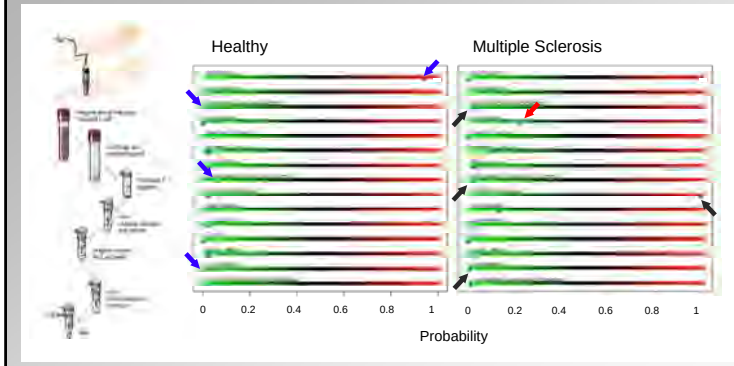
miR



Bauer *et al.* (2012) *PLoS ONE* **7**, e34151.

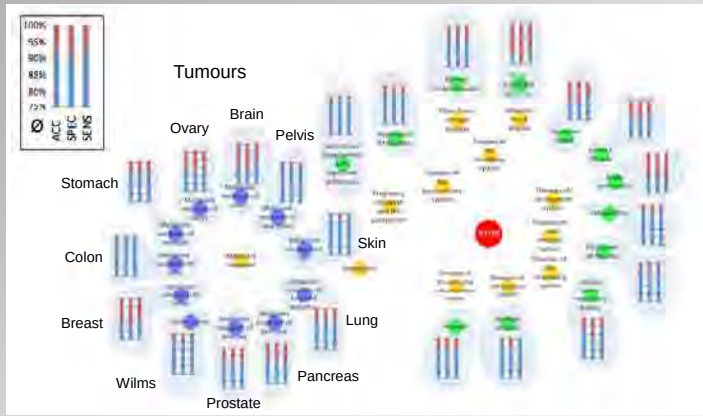
MicroRNA Diagnosis from Blood

miR



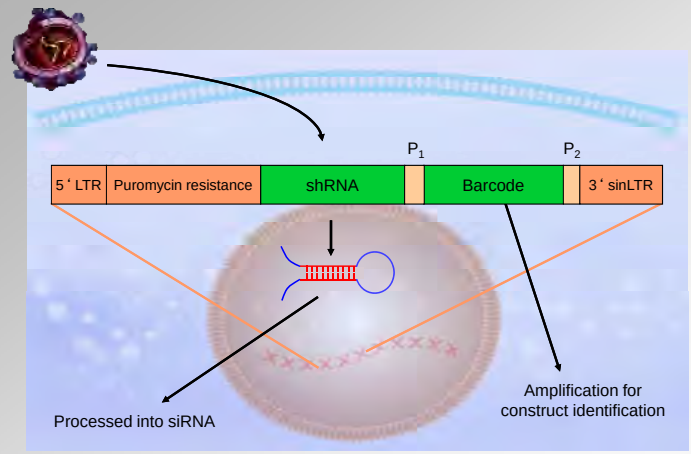
Keller *et al.* (2011) *Nature Meth.* **8**, 841-843.

MicroRNA Diagnosis from Blood



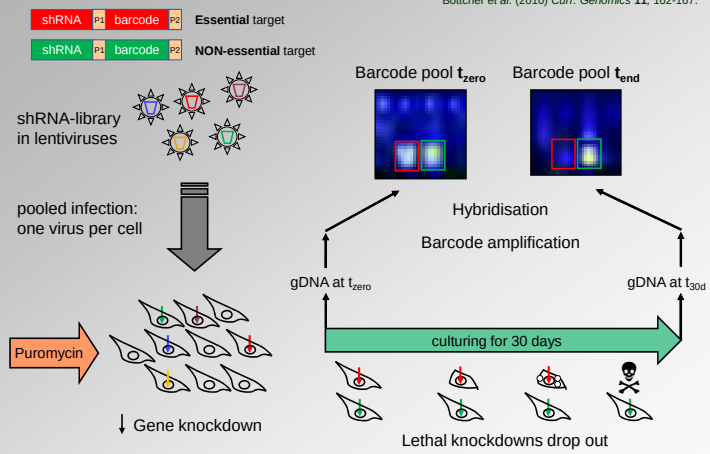
Keller *et al.* (2013) *Genome Biol.*, submitted.

Stable RNAi by Lentiviral Transduction

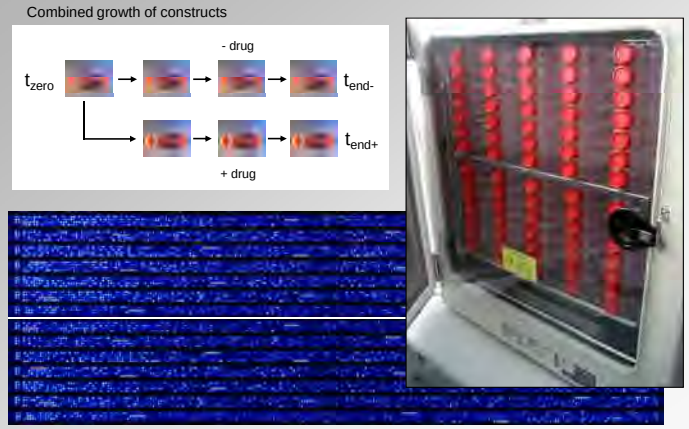


Global shRNA Screening

Böttcher *et al.* (2010) *Curr. Genomics* **11**, 162-167.

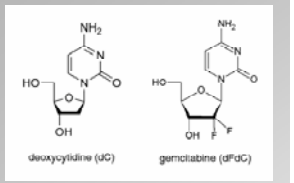


Synthetic-Lethal Screen

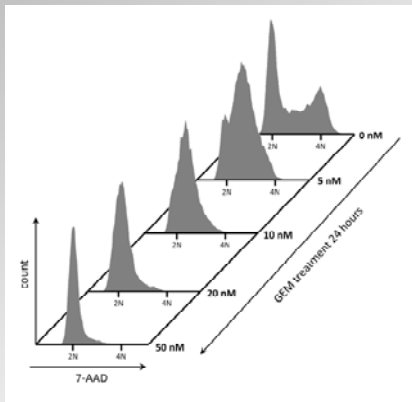


Böttcher *et al.* (2010) *BMC Genomics* **11**, 7.

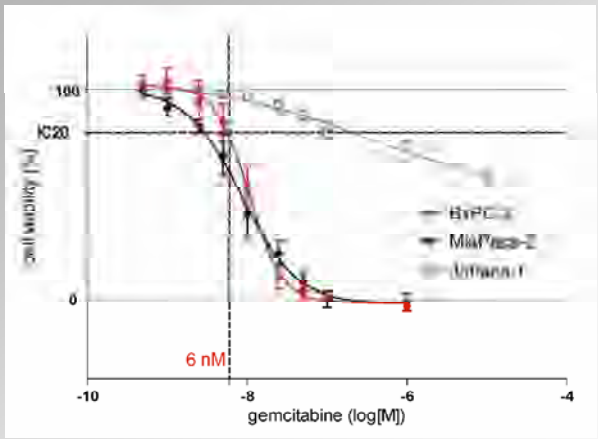
Improving the Effect of Gemcitabine



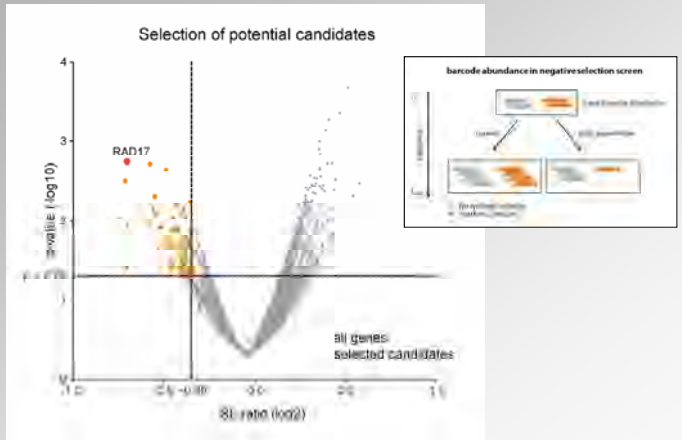
Inhibition of replication



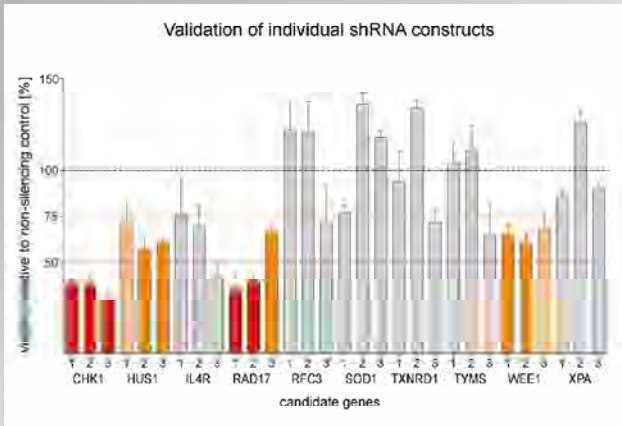
IC_{20} of Gemcitabine



Result of Synthetic-Lethal Screen

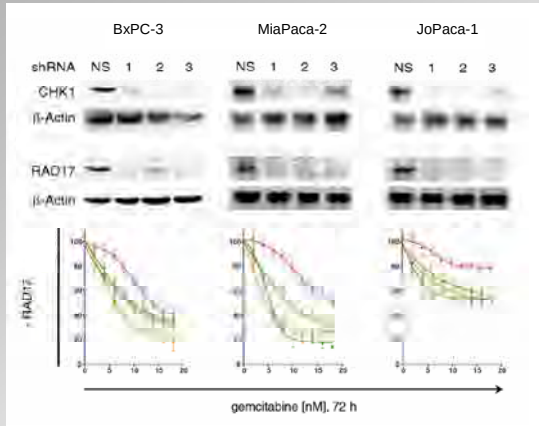


Validation of Screen Result

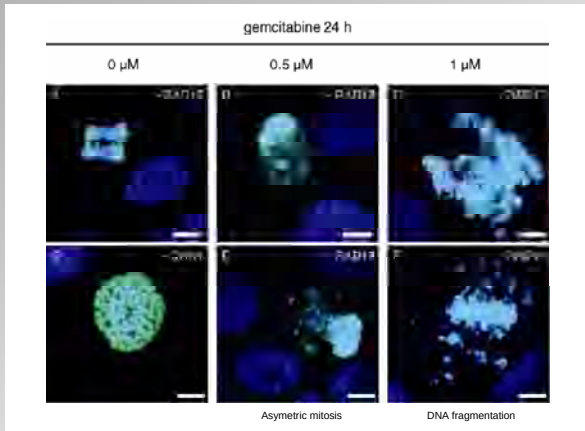


BxPC-3 treated with 6 nM gemcitabine für 72 h

Validation of RAD17

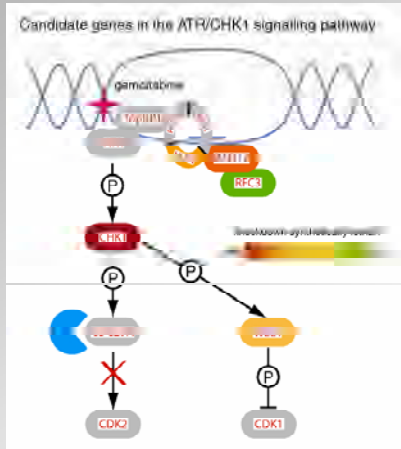


Elucidation of Mechanism



Fredebohm *et al.* (2013), *J. Cell Sci.*, in press.

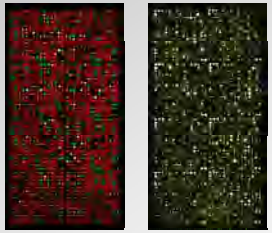
Elucidation of Mechanism



Fredebohm *et al.* (2013), *J. Cell Sci.*, in press.

Protein Expression & Structural Profiling

Antibody Microarrays



Goat anti-mouse IgG
Goat anti-rabbit IgG

Dual colour incubation with two serum samples

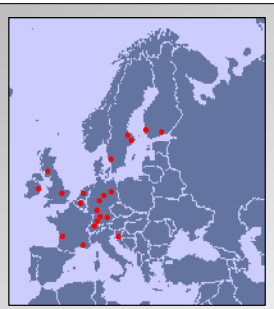
Alhamdani *et al.* (2010) *J. Prot. Res.* **9**, 963-971.
Gloriam *et al.* (2010) *Mol. Cell. Prot.* **9**, 1-10.
Schröder *et al.* (2010) *Mol. Cell. Prot.* **9**, 1271-1280.
Alhamdani *et al.* (2010) *Proteomics*, **10**, 3203-3207.
Schmidt *et al.* (2011) *J. Prot. Res.* **10**, 1316-1322.
Mustafa *et al.* (2011) *Mol. Biosyst.* **7**, 1795-1801.
Friedrich *et al.* (2011) *Proteomics* **11**, 3757-3760.
Schröder *et al.* (2011) *Meth. Mol. Biol.*, 203-221.
Alhamdani *et al.* (2012) *J. Proteomics* **75**, 3747-3759.
Hoheisel *et al.* (2013) *Prot. Clin. Appl.*, in press.

Binder Molecules

Antibody Microarrays

EU binder projects

PROTEOMEBINDERS AFFINITYPROTEOME AFFINOMICS



Taussig *et al.* (2007) *Nature Meth.* **4**, 13-17.
Gloriam *et al.* (2010) *Mol. Cell. Prot.* **9**, 1-10.

Transcriptional Profiling

- DNA sequences of 900 genes/ESTs exhibiting differential transcription
- Peptide selection from sequences according to 12 selection criteria
- Peptide synthesis
- Immunisation of rabbits
- Affinity purification of polyclonal antibodies
- Characterisation by *in situ* analyses

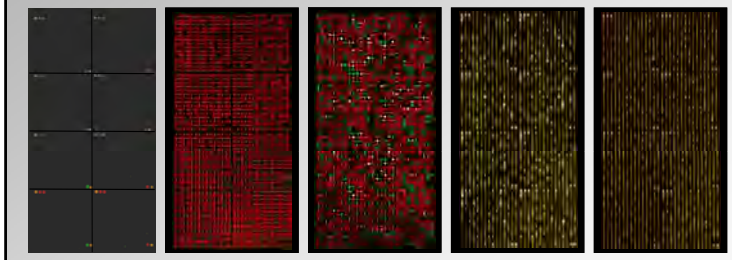
Expression Profiling

Current Chip Layout

Antibody Microarrays

810 different antibodies

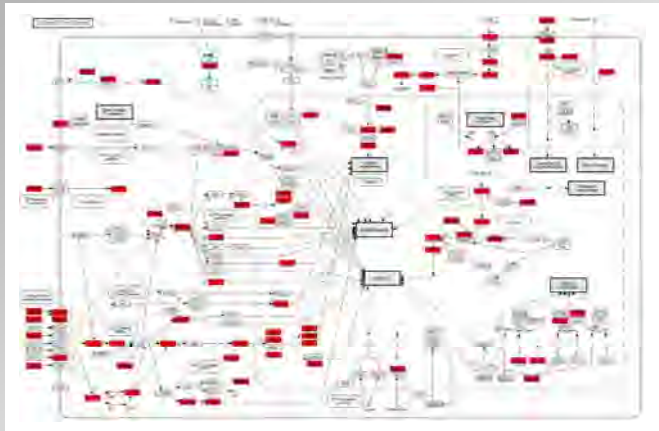
678 selected based on significant variations in transcriptional studies
67 additional keyplayers in cancer-related pathways
40 from literature research and collaboration partners
25 housekeeping proteins and controls



Positional marker proteins | Protein labelling with Sypro Ruby | Goat anti-mouse IgG / Goat anti-rabbit IgG | Human serum samples | Human urine samples

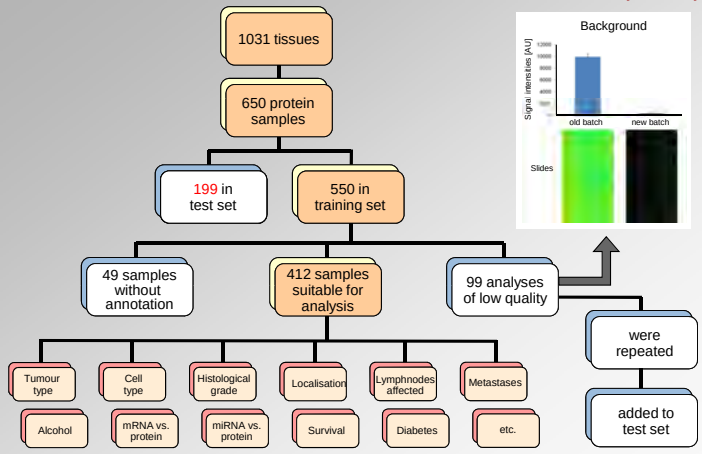
Cancer Binders

Antibody Microarrays



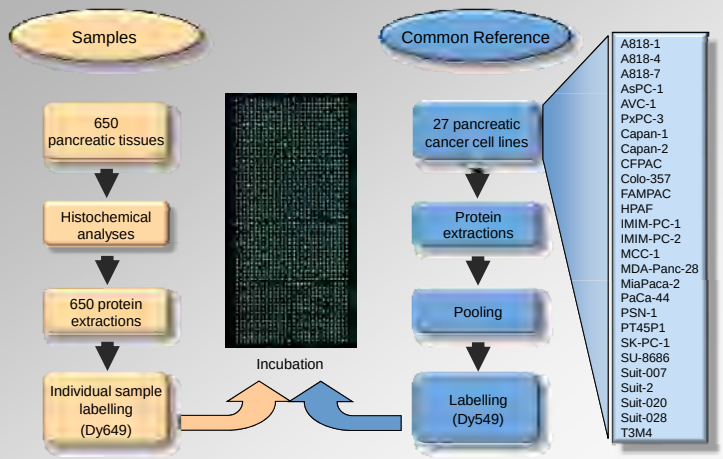
Profiling Pancreatic Cancer Tissues

Antibody Microarrays



Dual Colour Modus

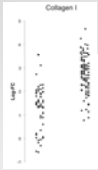
Antibody Microarrays

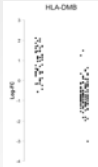


Profiling Pancreatic Cancer Tissues

Antibody Microarrays

Classification based on Histology (HE-staining)	No. patients	No. regulated proteins	No. specific proteins
Normal	56	-	-
Normal (inflammation)	19	207	20
Normal (cancer)	97	415	18
Inflammation	70	325	17
Inflammation (cancer)	13	96	0
PDAC	105	475	35
Cystic tumours	25	343	2
Endocrine tumours	16	432	20
Acinar cell carcinoma	2	50	0
Adenocarcinoma	9	200	2
Total	412		





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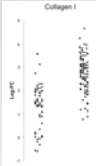
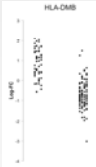
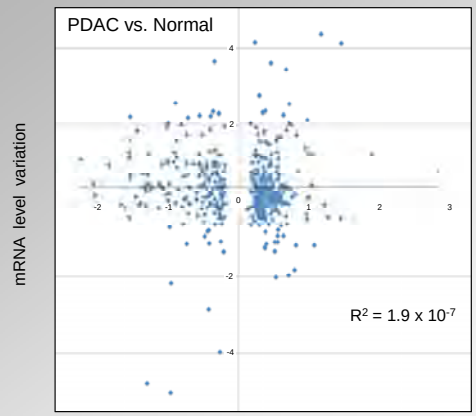




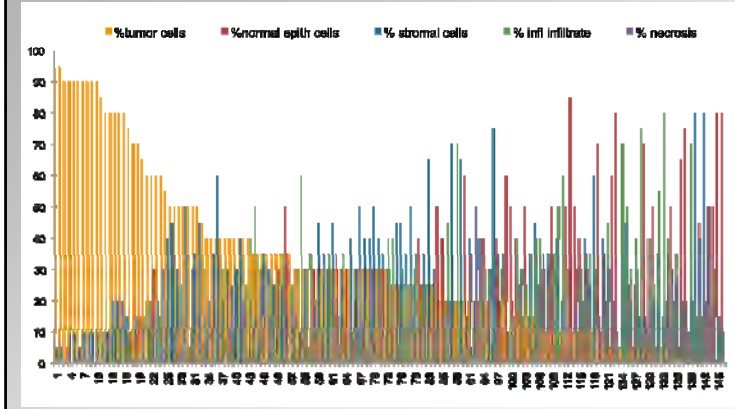
Correlation Protein to mRNA

Antibody Microarrays



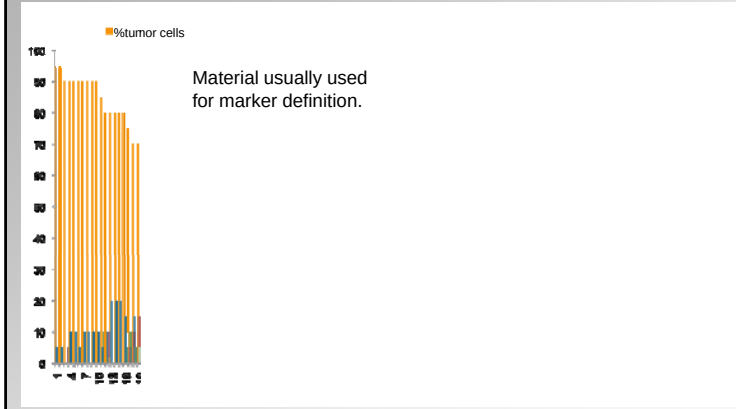
Profiling Pancreatic Adenocarcinoma

Antibody Microarrays



Profiling Pancreatic Adenocarcinoma

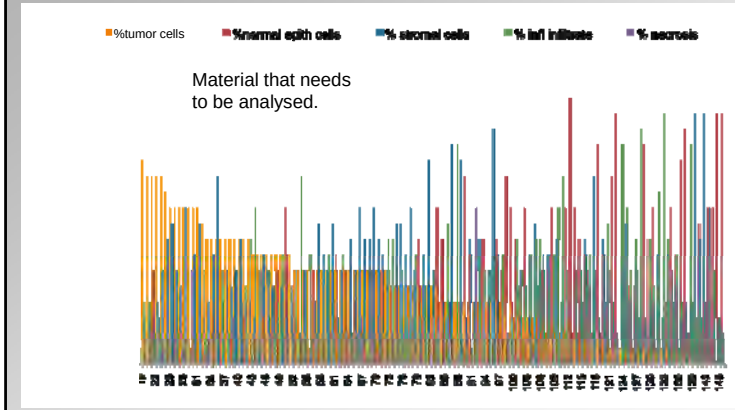
Antibody Microarrays



Material usually used
for marker definition.

Profiling Pancreatic Adenocarcinoma

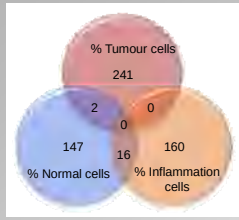
Antibody Microarrays



Material that needs
to be analysed.

Correlation with Cell Type

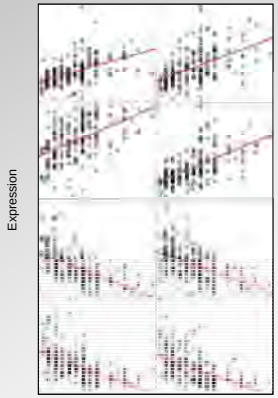
Antibody Microarrays



Directly correlated proteins

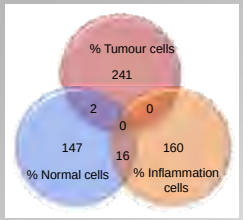
Cell type	+ve correlated	-ve correlated	total
% Cancer cells	155	88	243
% Normal cells	38	127	165
% Inflam. cells	84	92	176
% Stromal cells	148	94	242
% Necrotic cells	76	79	155

➡ Classifier of tissue composition
=> Molecular Pathology



Tumour Cell and *Tumour Tissue* Specificity

Antibody Microarrays



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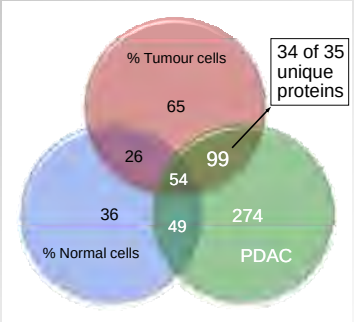
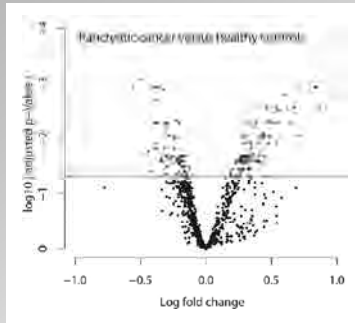


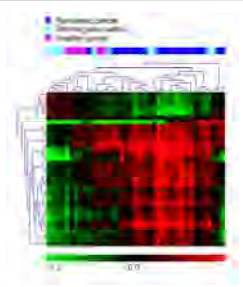
Optimal PDAC markers

Non-Invasive Diagnosis: Plasma

Antibody Microarrays

Differentiation PDAC *vs.* Healthy & Chronic Pancreatitis: Specificity 76%
Sensitivity 77%

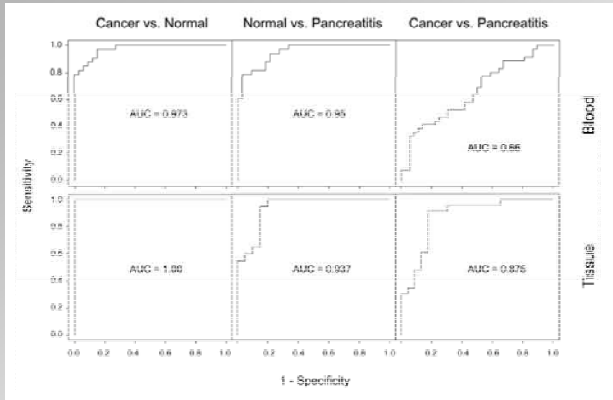




Cluster analysis of
PAM-defined classifier proteins

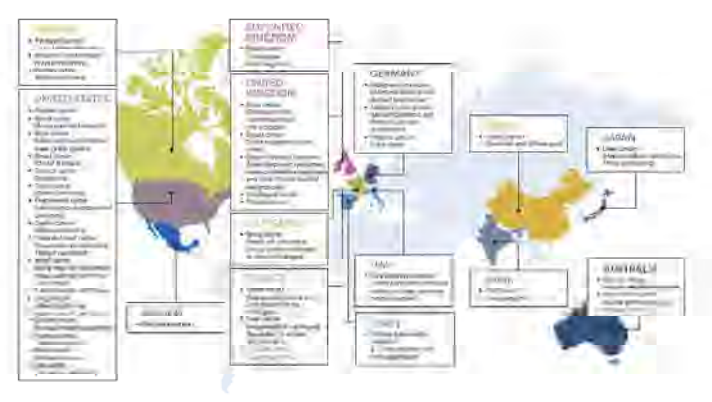
MicroRNA Diagnosis from Blood

miR



Bauer *et al.* (2012) *PLoS ONE* **6**, e27921.
Keller *et al.* (2011) *Nature Meth.* **8**, 841-843.

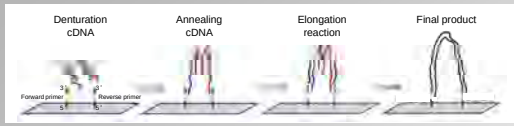
International Cancer Genome Consortium

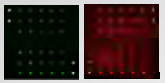


Presentation of a Personal Proteome

Personalised Proteomics

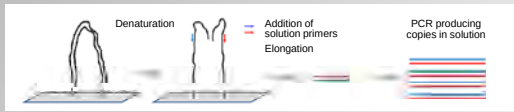
Solid-phase PCR on cDNA of individual tissue sample





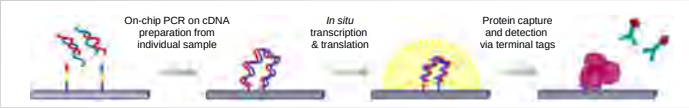
Hybridisation with probe complementary to plus (green) or minus (red) strand of DNA

Solution-phase PCR for length determination



PCR-fragments of up to 4 kb could be amplified.

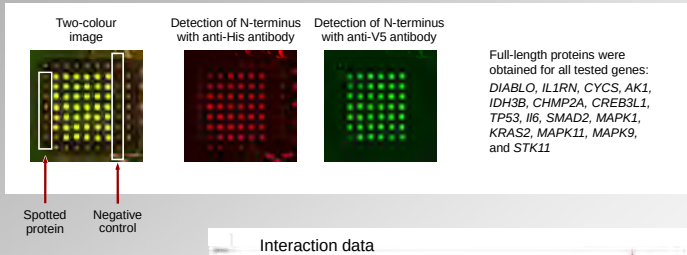
Representation of personalised proteome



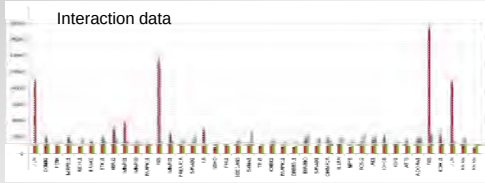
Presentation of a Personal Proteome

Personalised Proteomics

Check for full-length proteins by detection of terminal tag molecules



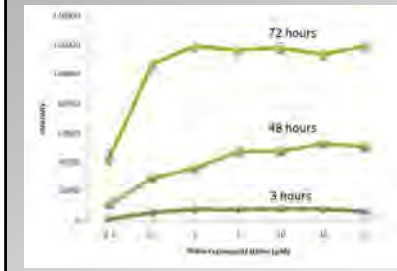
Full-length proteins were obtained for all tested genes: *DIABLO, IL1RN, CYCS, AK1, IDH3B, CHMP2A, CREB3L1, TP53, Il6, SMAD2, MAPK1, KRAS2, MAPK11, MAPK9,* and *STK11*



Production Parameters

Personalised Proteomics

Optimal primer concentration and immobilisation time



Full length on-chip PCR products

ICAM3: 3561 bp

SORT1: 2493 bp

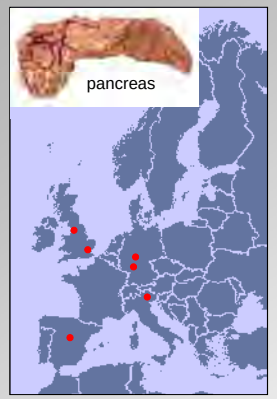
CREB3L1: 1557 bp

IL1RN: 477 bp

Semiquantitative comparison

The amount of bound DNA is equivalent to amount achieved by spotting PCR products

Studying Pancreatic Cancer



Nathalia Giese, Jens Werner & Markus Büchler
Surgery Department
University Clinics Heidelberg

Eithne Costello, William Greenhalf & John Neoptolemos
Surgery and Oncology
Royal Liverpool University Hospital

Aldo Scarpa
Pathology Department
University of Verona

Malte Buchholz & Thomas Gress
Gastroenterology & Endocrinology
University of Marburg

Tatjana Crnogorac-Jurcevic
Molecular Oncology and Imaging
Queen Mary Hospital,
University of London

Núria Malat & Francisco Real
Molecular Pathology & Epidemiology
Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid

Division of *Functional Genome Analysis*, DKFZ, Heidelberg

www.dkfz.de/funct_genome

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