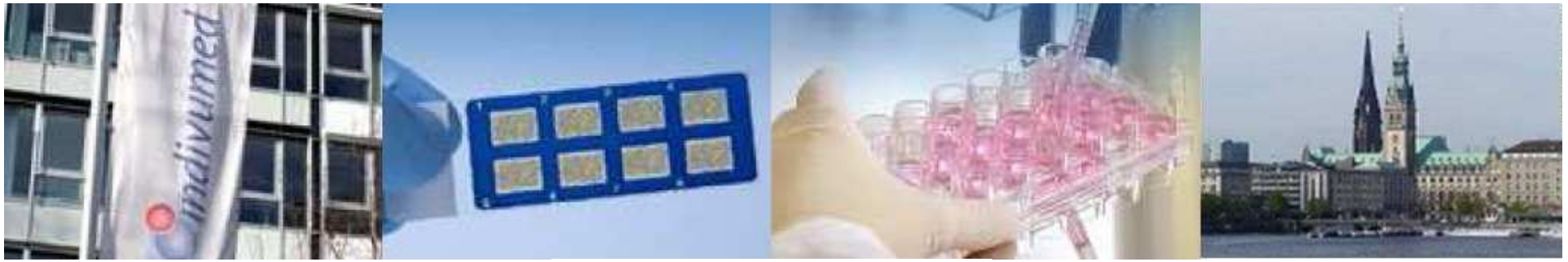

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Translating Science to Personalized Medicine

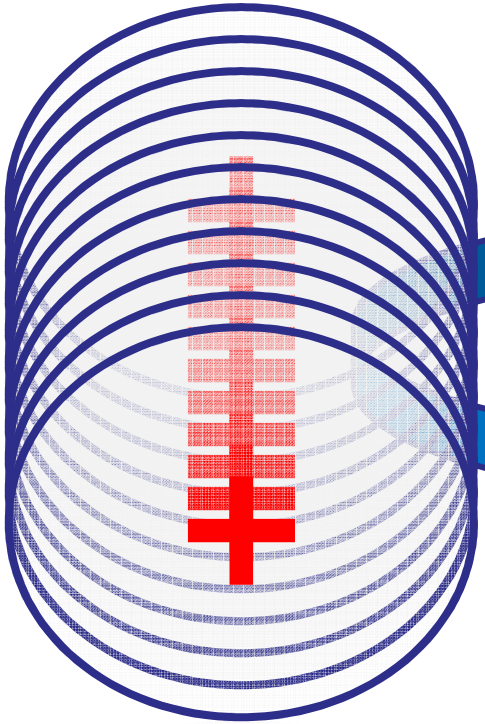
Der Aufwand zur Qualitätssicherung bestimmt den wissenschaftlichen Nutzen einer Biobank

Prof. Dr. Hartmut Juhl
CEO of Indivumed GmbH and IndivuTest GmbH
Adjunct Professor, Georgetown University and Hamburg University
www.indivumed.com
juhl@indivumed.com

Overview Indivumed Groupe

Founded:	September 2001, operative start April 2002
Shareholders:	Founder & private investors
Employee:	75
Locations:	Hamburg, Germany (Indivumed GmbH, Indivutest GmbH) Washington DC (Indivumed Inc);
Business Field:	Oncology: Products & Services for biomarker / drug development and patient care
Customers:	Pharmaceutical/Biotech Industry, Academia, Oncologists/Patients
Clinical Partner:	Hamburg: 9 Cancer Clinics and 10 Oncology Practices Washington DC: Georgetown University Medical Center & Washington Hospital Center Danville, PA: Geisinger Health System
Academic Partner:	Ludwig Institute at Kimmel Cancer Center, Johns Hopkins University Lombardi Cancer Center, Georgetown University University Clinic of Hamburg US-National Cancer Institute Stanford University Food and Drug Administration USA

Indivumeds Approach:
Integration of Research, Surgery and Patient Care



Partner Hospitals

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Indivumed Clinical Team

Indivumed Scientific Team

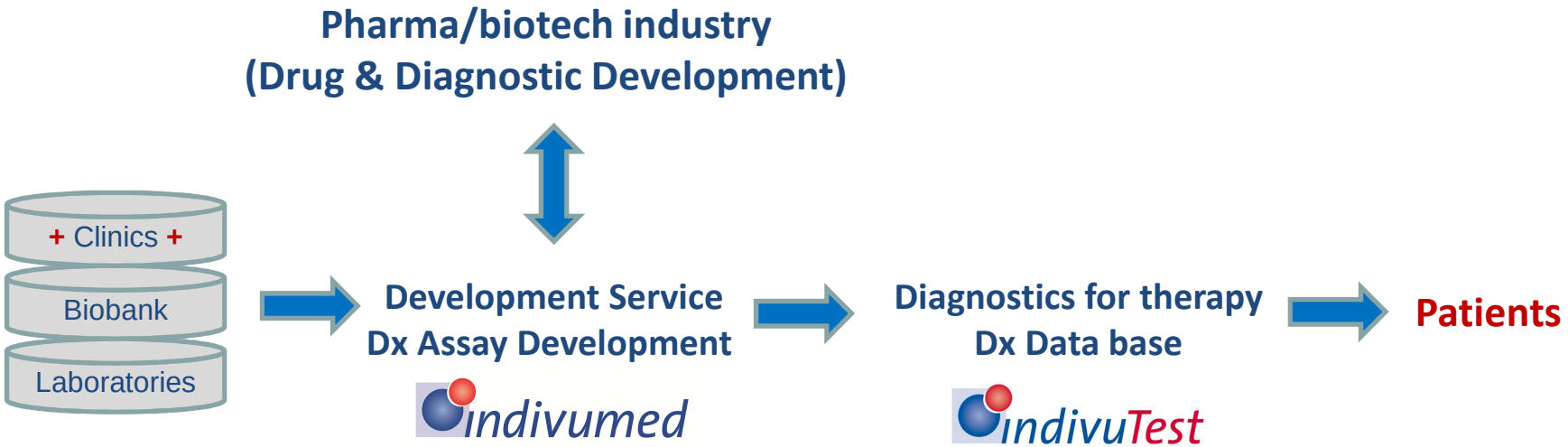
Biospecimen Direct Processing

Biospecimen
- Tumor tissue
- Normal Tissue
- Blood
- Urine

Data
- Processing Data
- Clinical Data
- Follow-up Data

Tumor-Biobank and Clinical Data Base

Indivumed business field



Business Areas:

1. Fee-for-Service

- Preclinical Development
- Clinical trial support
  - Tissue collection
  - Tissue analysis

2. Development Partner

- Target discovery & validation
- Diagnostic Assay Development

Patient diagnostics:

- Testing for individualized therapy
- Diagnostic data base for future developments

Tumor-Biobanking:
to discover and validate findings for clinical use

DNA → **mRNA** → **Proteins** → **Protein Modifications (e.g., phosphorylation)**

Mutations
KRAS
PIK3CA
BRAF
EGFR
Many others

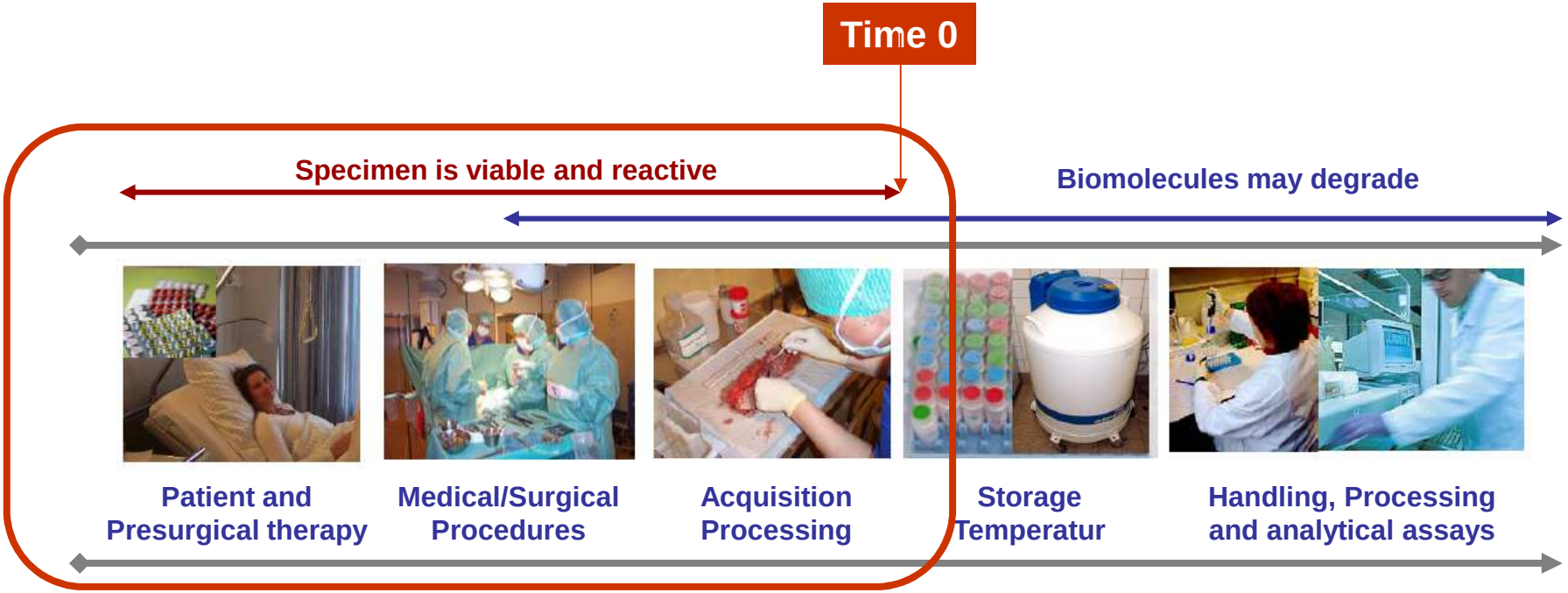
Gene Expression
Oncotype
AmpliChip
MammaPrint
Others

Target-Expression
HER-2
EGFR
VEGFR
Others
Protein Profiling

Functionality
pmTOR
P-AKT
P-MAPK
Many, many more

Clinical value and Importance of tissue quality

Indivumeds Core Competence:
To Solve the Quality Challenges of Tissue Based Research



Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing

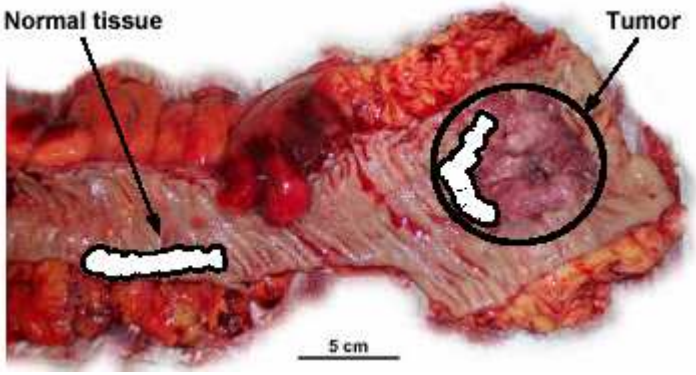
Impact of Cold Ischemia: Controlled Tissue Study

Surgical removal

of rectum

Control of warm ischemia

Collection of normal and cancer tissue



Tissue collection following resection:
Snap frozen in liquid N2
→ after 5 min
8 min
10 min
12 min
15 min
20 min
25 min
30 min

Analysis:

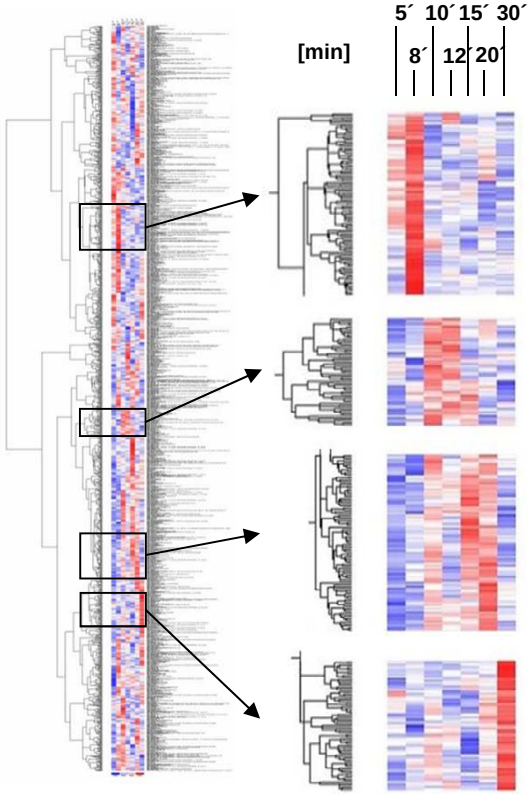
Affymetrix

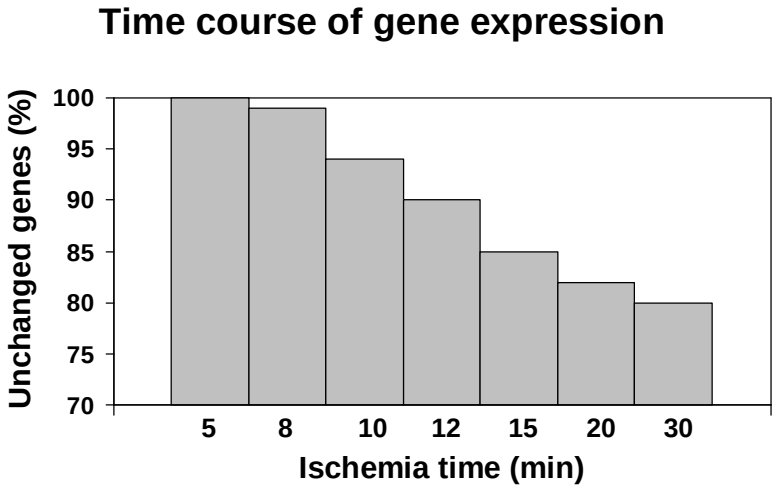
real-time RT-PCR

SELDI-TOF-MS

Tissue Ischemia and Gene Expression Profiling

(Affymetrix cDNA microarray)





Following tumor resection ~ 20-25% of genes are differentially expressed within the first 30 minutes !

Sprüssel et al, BioTechniques 2004

Research Studies on the Effect of Intra- and Post-operative Ischemia on Gene and Protein Expression Patterns in Liver (Project 1) and Colorectal Tissue (Project 2).
An Exploratory Research Study (29XS111)

Funded by NCI Contract No. HHSN261200800001E

Partner:

OBBR/NCI

Indivumed GmbH

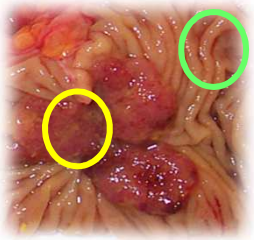
Department of Surgery, Israelitisches Krankenhaus (Dr. Zornig)

Department of Surgery, Diakonieklinikum Alten Eichen (Dr. Dörner)

Department of Hepatobiliary Surgery, University Hospital Hamburg (PI: Dr. Nashan)

Impact of anesthesia and surgery on gene and protein expression in colon and liver tissue : Study design

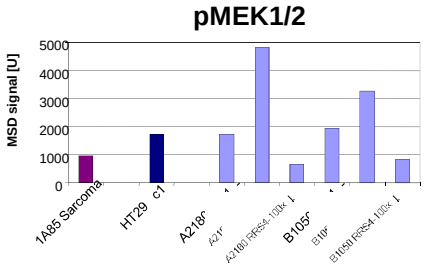
Cancer



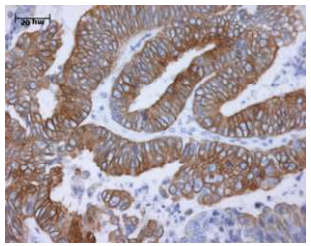
Normal

Cancer-relevant Proteins

Protein Quantification

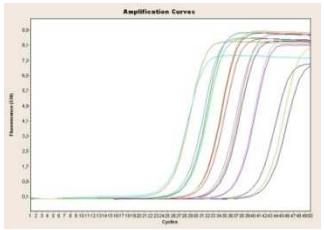


Cellular Distribution

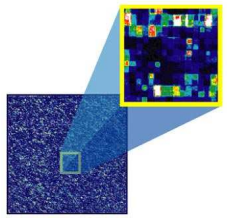


Gene Expression Profiling

Specific Genes

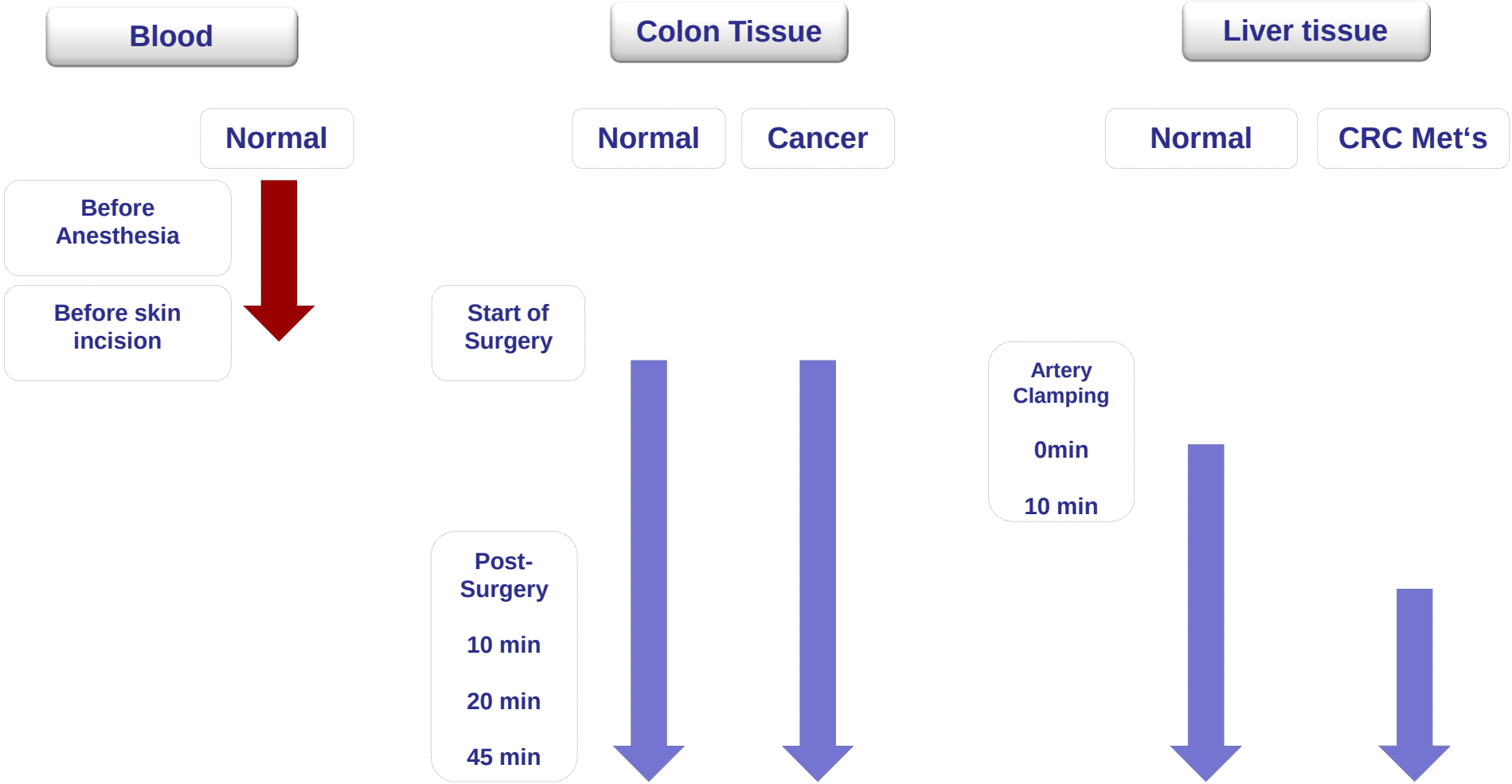


Comprehensive Analysis



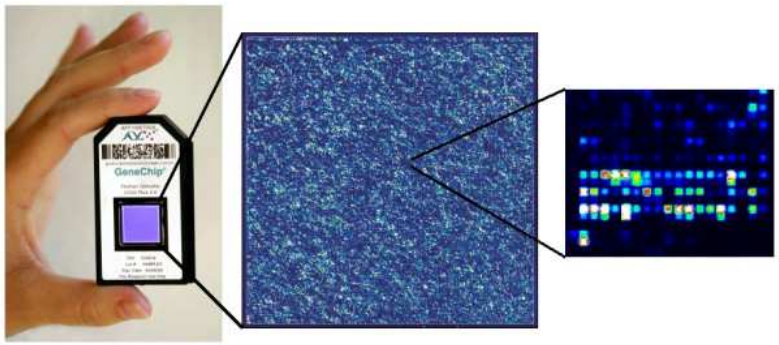
Identification of Surgery Dependent Molecules

Impact of anesthesia and surgery on gene and protein expression in colon and liver tissue



Gene expression analysis:
Affymetrix whole genome array (Human Genome U133 Plus 2.0)

- Preparation of RNA RIN >7
- Chip analysis in biological replicates
- Statistical and bioinformatic analysis of data for
    - Each case time course
    - All cases time course
    - Comparison tumor/normal
    - Comparison with protein data



Analysis of proteins: MSD technology

Frozen Tissue: MSD analysis

- pEGFR/total EGFR
- pMEK1/2/total MEK1/2 (Ser217/221)
- pERK1/2/total ERK1/2 (Thr202/Tyr204, Thr185/Tyr187)
- pAkt/total Akt (Ser473)
- pmTOR/total mTOR (Ser 2448)
- pP70S6K/total P70S6K (Thr421/Ser424)
- pGSK-3beta/total GSK-3beta (Ser9)
- pHSP27/HSP27
- Hif-1alpha

Analysis of proteins: Immunohistochemistry

FFPE Tissue
IHC (Ventana)

- pEGF-R
- pHER-3
- pMAPK
- pAKT
- pmTOR

The H-Score was calculated as follows:

H-Score = 3x strongly stained tumor cells (%)
+ 2x moderately stained tumor cells (%)
+ 1x weakly stained tumor cells (%)

The H-Score categories:

- **0 - 50 negative**
- **51 – 100 weak positive**
- **101 – 200 moderate positive**
- **201 – 300 strong positive**

n=50 / full set = 2 vials

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Impact of anesthesia and surgery on gene and protein expression in colorectal and liver tissue

Liver and CRC metastases samples

time (min)	normal (cryo)	normal (formalin)	tumor (cryo)	tumor (formalin)
before ligation	40/43	33/43	n/a	n/a
10‘ after ligation	36/43	34/43	n/a	n/a
Post 10’	40/43	39/43	42/43	39/43
Post 20’	40/43	37/43	42/43	40/42
Post 45’	40/43	36/43	41/42	38/43

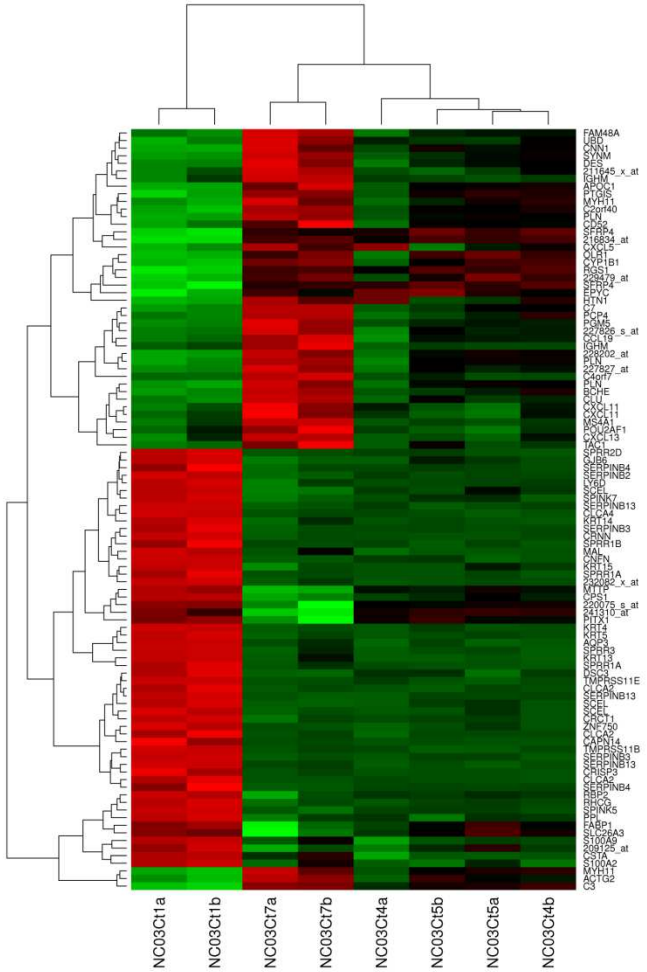
Colon and CRC tumor samples

time (min)	normal (cryo)	Normal (formalin)	tumor (cryo)	tumor (formalin)
Endoscopy	48/50	47/50	48/50	47/50
Post 10’	49/50	49/50	49/50	43/50
Post 20’	49/50	49/50	49/50	43/50
Post 45’	49/50	49/50	49/50	43/50

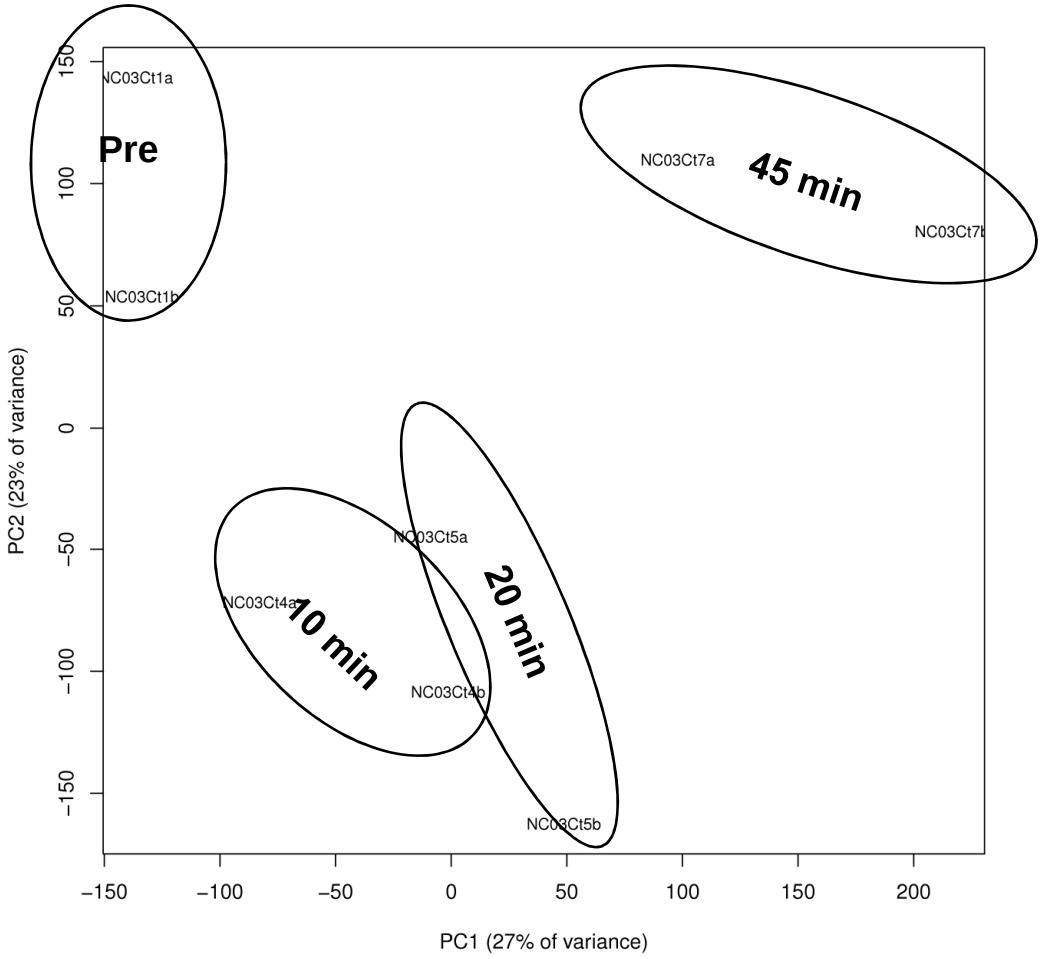
Impact of intra- and postoperative Factors on Tissue Analysis : Gene Expression (Affymetrix-Analysis) in CRC

(NCI-Indivumed Studie, 2012)

Heatmap of top 100 differential expressed genes

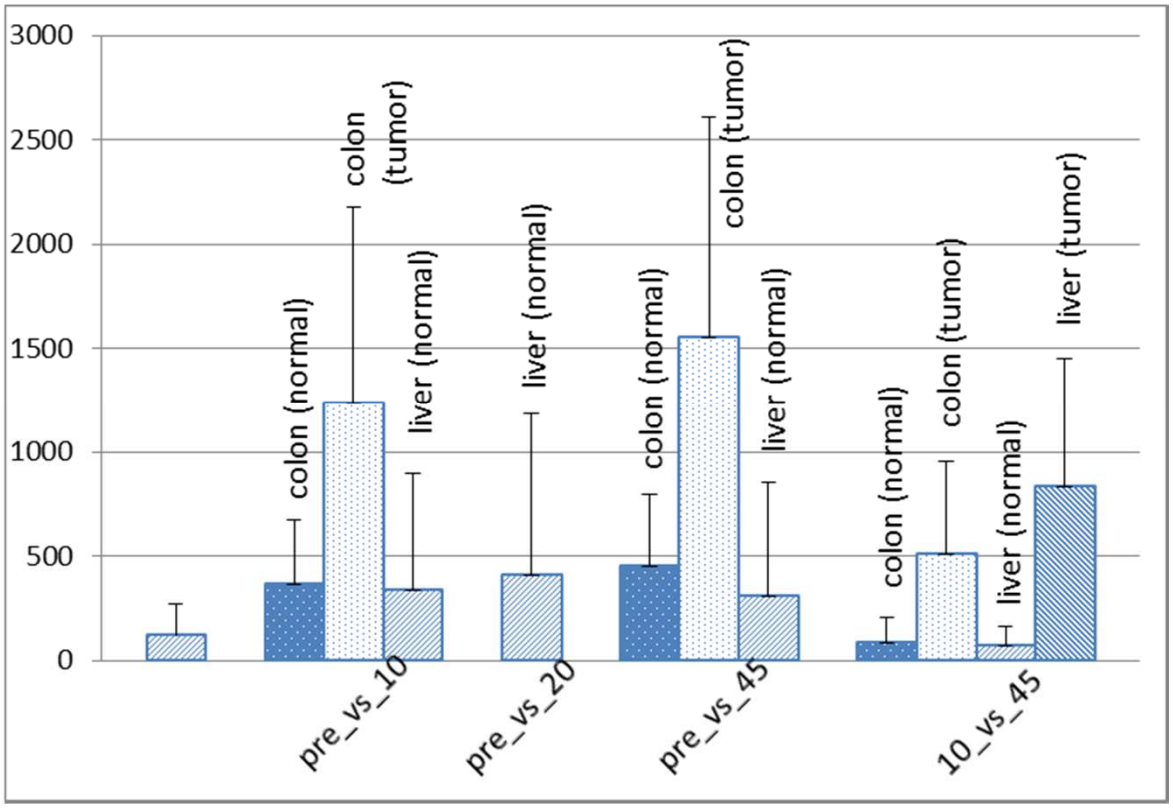


Principal Component Analysis (PCA)

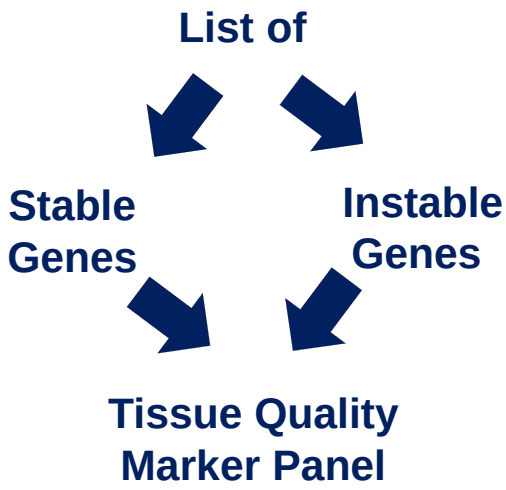


Impact of intra- and postoperative Factors on Tissue Analysis : >2-fold change of gene expression

(NCI-Indivumed Studie, 2012)

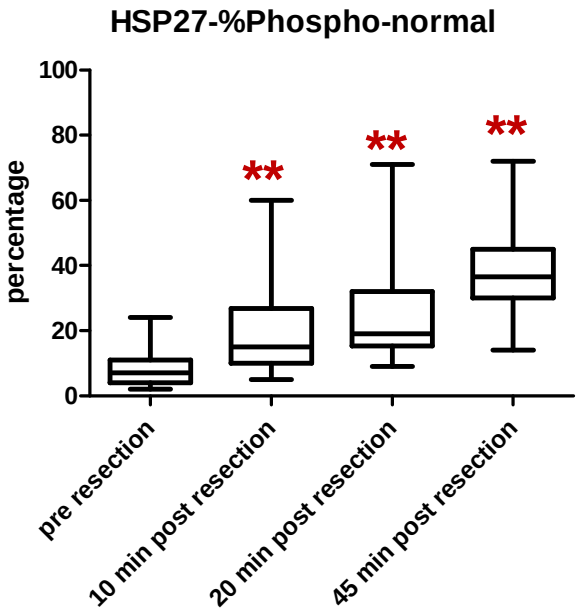


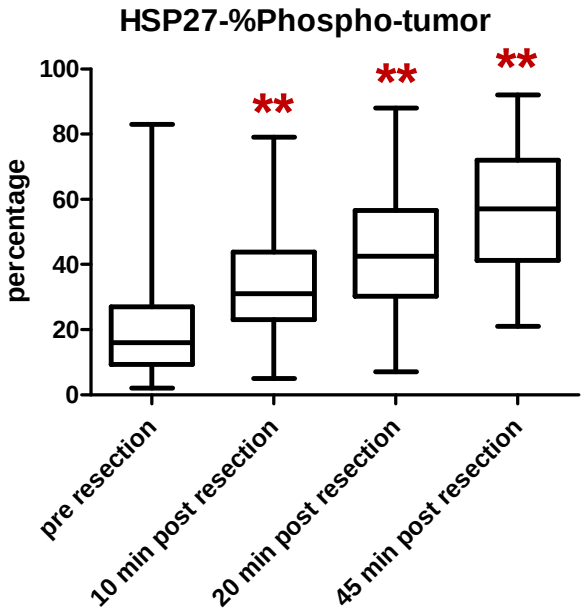
Expression of up to 20% of genes change during surgery and postsurgical processing time >2x



Impact of intra- and postoperative Factors on Tissue Analysis : Example phospho-HSP27 (MSD-Analytic) in CRC

(NCI-Indivumed Studie, 2012)

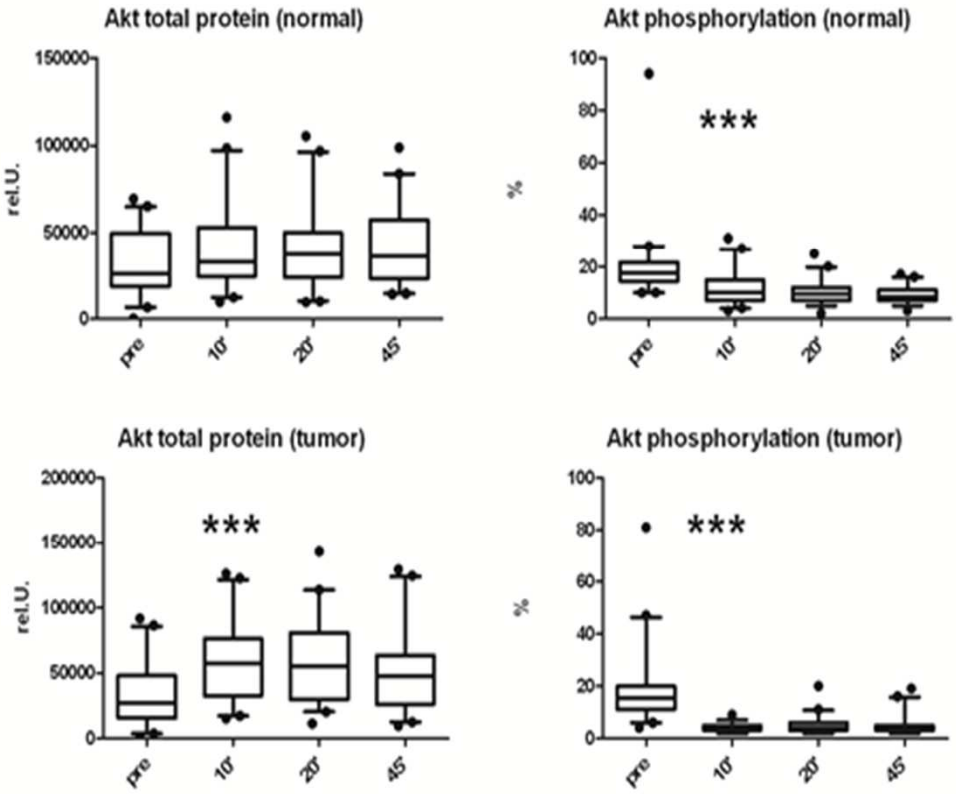


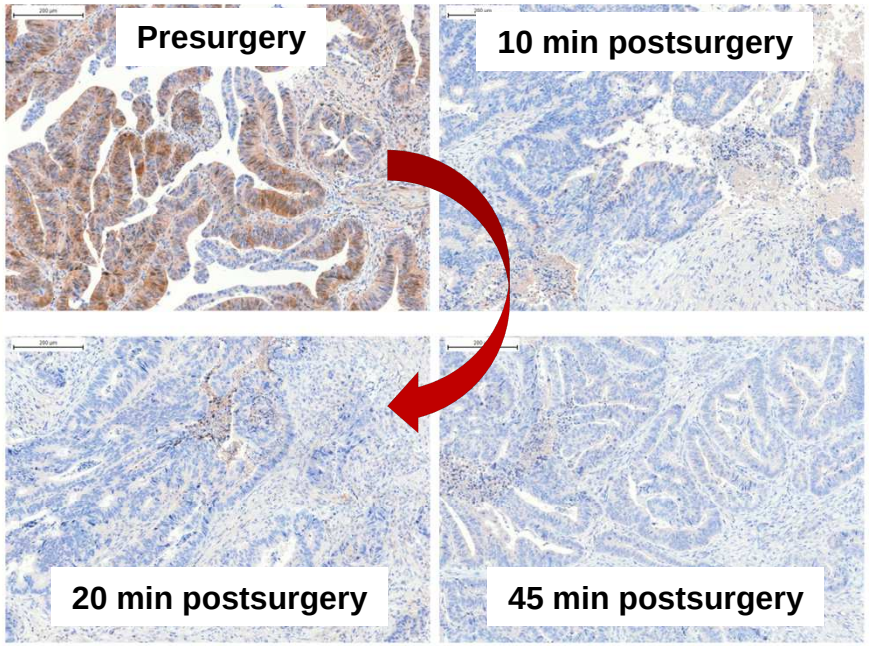


**Significantly affected

Impact of intra- and postoperative Factors on Colon Tissue Analysis by Immunohistochemistry (pAKT):

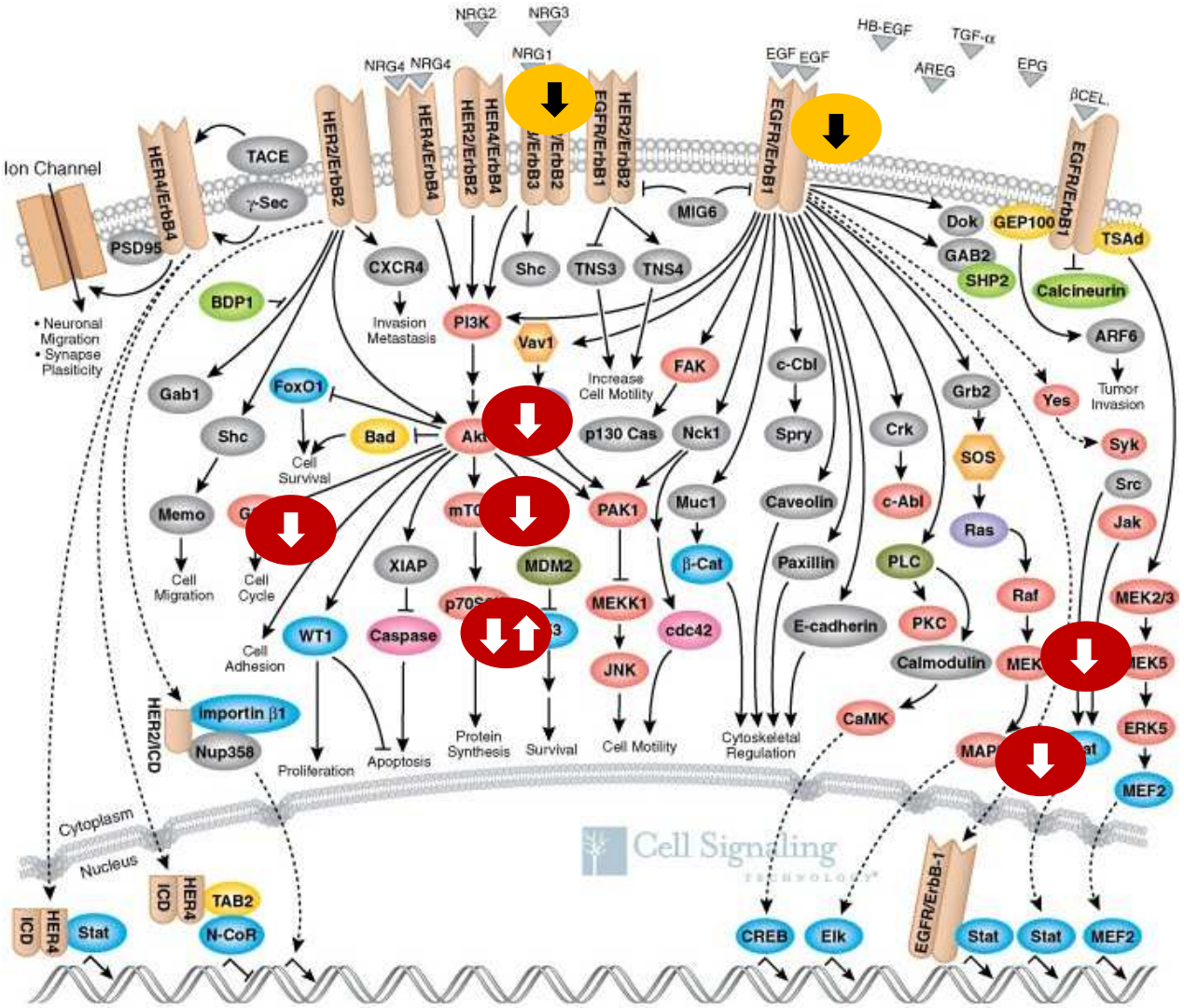
(NCI-Indivumed Studie, 2012)





Change of EGFR-pathway activity by surgical manipulation and tissue processing in colorectal cancer tissue

(NCI-Indivumed Studie, 2012)



significant

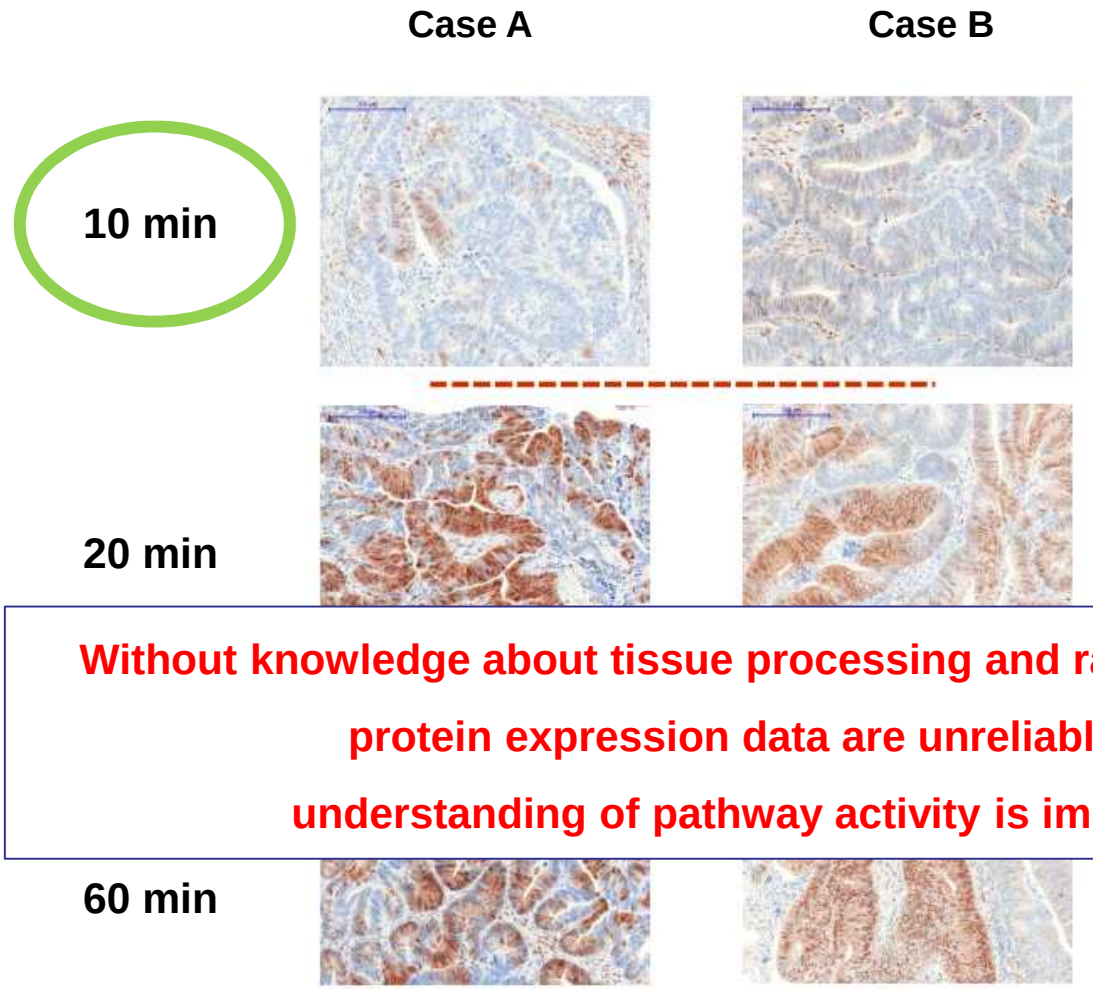
Only MSD

Not significant

Comparison:

Tumor biopsy
--
10 min postsurgery
-
45 min postsurgery

Phosphoprotein Expression: pMAPK Immunostaining (Ventana)



Without knowledge about tissue processing and rapid tissue fixation protein expression data are unreliable and understanding of pathway activity is impossible

Conclusion

Rapid and highly controlled tissue collection is mandatory for obtaining clinically meaningful data based on:

- **Gene expression pattern**
- **Protein pattern**
- **Phosphoproteins**

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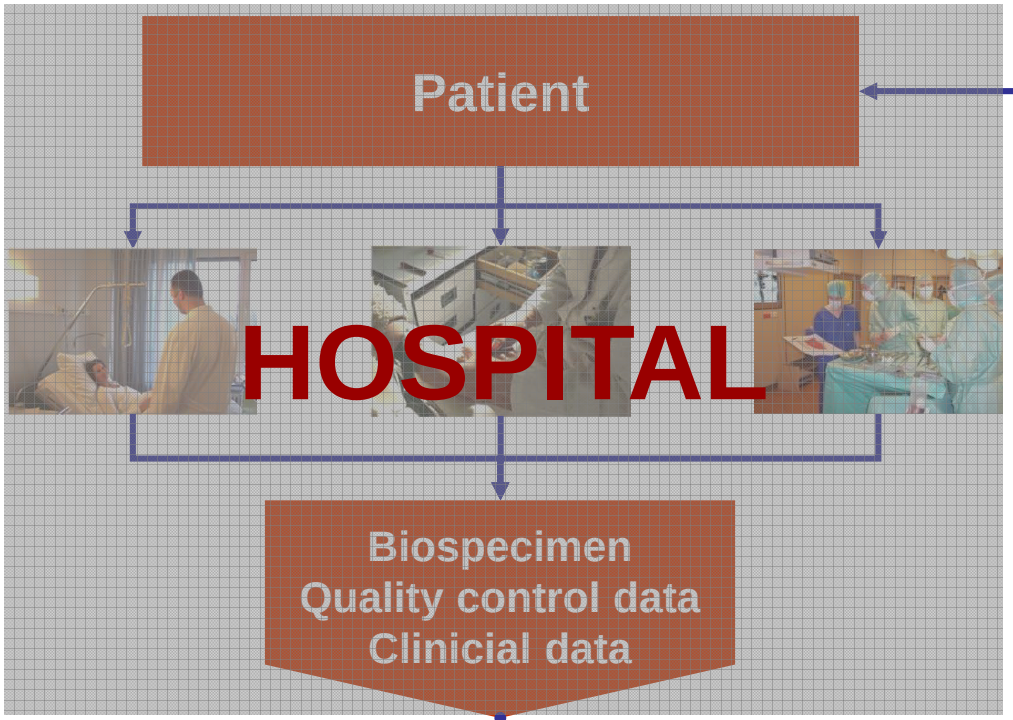
Tissue Collection for Research: A Black Box as Research Tool ?!?



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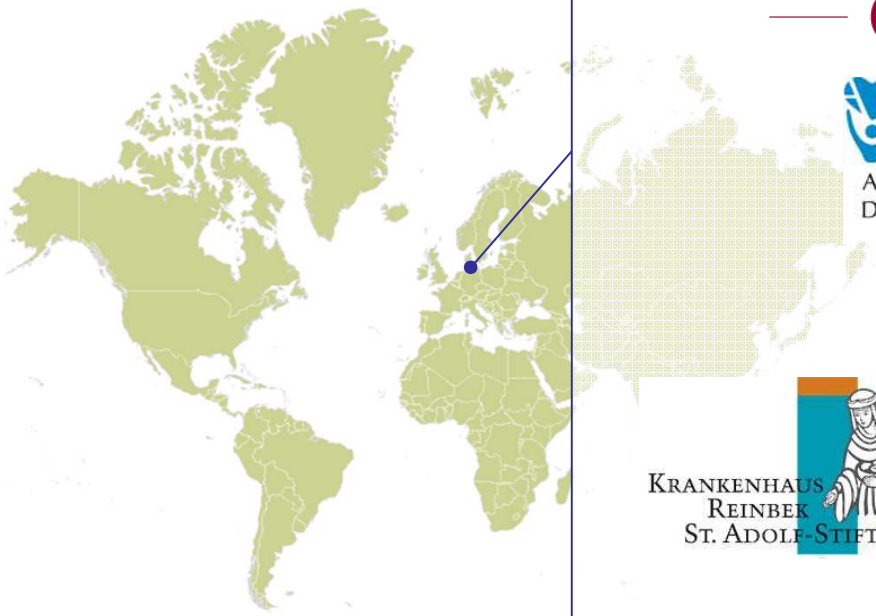
Basic Consideration for Achieving Science-Guided Bioanking:

Take responsibility away from clinical staff!
Biospecimen and data collection exclusively by specialized experts!

Indivumed‘s Network of Collaborating Hospitals



Hamburg, Germany

ISRAELITISCHES KRANKENHAUS IN HAMBURG

MARIEN KRANKENHAUS

AGAPLESION DIAKONIEKLINIKUM HAMBURG

Mammazentrum AM KRANKENHAUS JERUSALEM Hamburg

KRANKENHAUS REINBEK ST. ADOLF-STIFT

ASKLEPIOS Klinik Harburg

LUNGENCLINIC GROSSHANSDORF

Universitätsklinikum Hamburg-Eppendorf

and others

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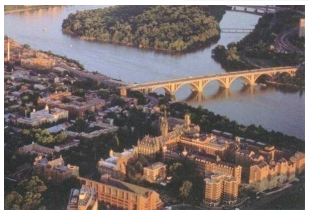
Indivumed‘s Network of Collaborating Hospitals

Washington, DC



Georgetown University Hospital
MedStar Health

Washington Hospital Center
MedStar Health

Danville, PA

GEISINGER HEALTH SYSTEM

Hamburg, Germany

ISRAELITISCHES KRANKENHAUS IN HAMBURG

MARIEN KRANKENHAUS

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ASKLEPIOS Klinik Harburg

LUNGENCLINIC GROSSHANSDORF

Universitätsklinikum Hamburg-Eppendorf

and others

Indivumed Solution:
Integration of Clinical Care, Biobanking and Research

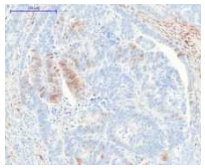
Tumor-Biobank and Clinical Data Base

- Collection sides in Germany and the US
- Identical biospecimen processing in all clinics
- Identical and comprehensive patient data
- All major tumor entities

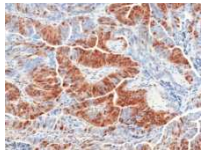
Indivumed Standard of Biobanking:

Tissue ischemia and protein phosphorylation

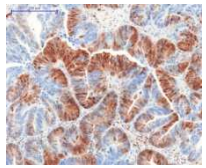
10 min



20 min



60 min



- ✓ **Exact documented and very short tissue cold ischemia times of < 12 min (mean 7 min)**
- ✓ **Exact tissue localization and standardized fixation**
- ✓ **Complete biospecimen sets**
- ✓ **Highest tissue quality monitored by visual inspection, H&E staining and microscopic assessment**
- ✓ **Native and rapid fluid preparations**
- ✓ **Complete specimen data**
- ✓ **Complete clinical data**
- ✓ **Patients' confidentiality assured following international standards**

Biobank:
Clinical Data and Biospecimen Data

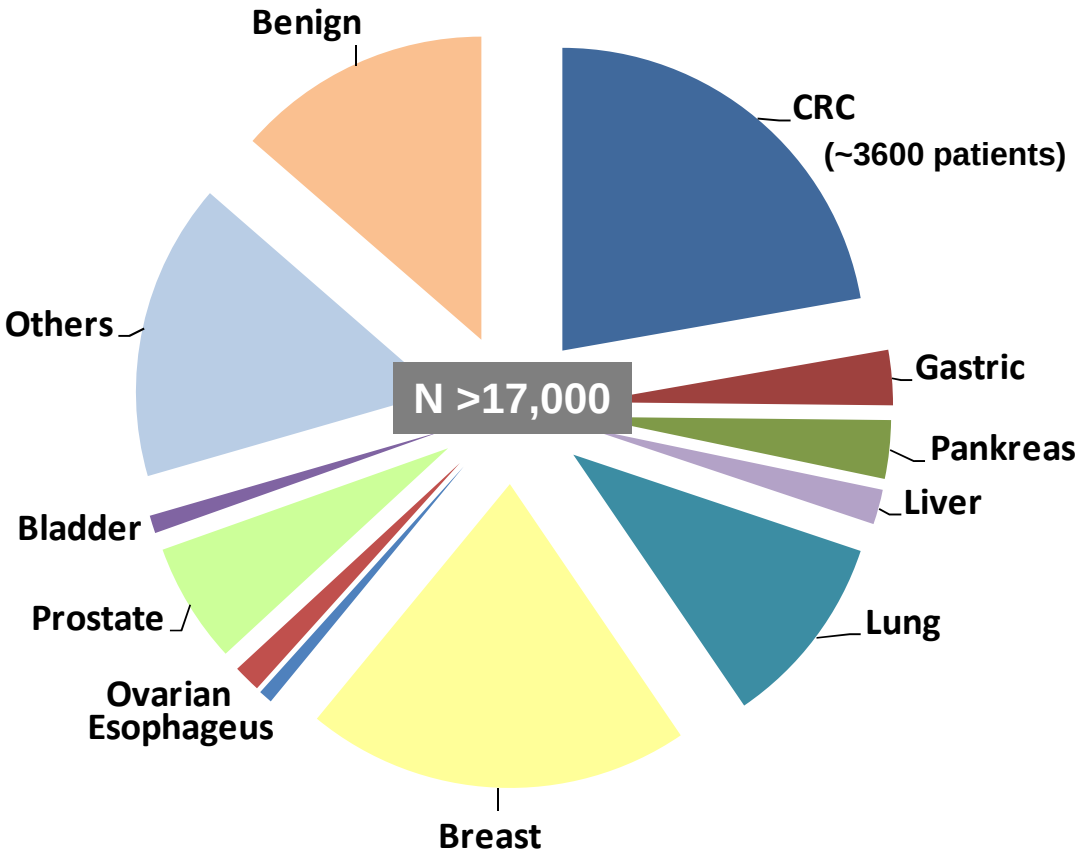
Clinical Data:

1. Medical History
2. Disease specific anamnesis
3. Diagnosis
4. Therapy
5. Follow-up
6. Comprehensive Lab-test Data

Biospecimen Data:

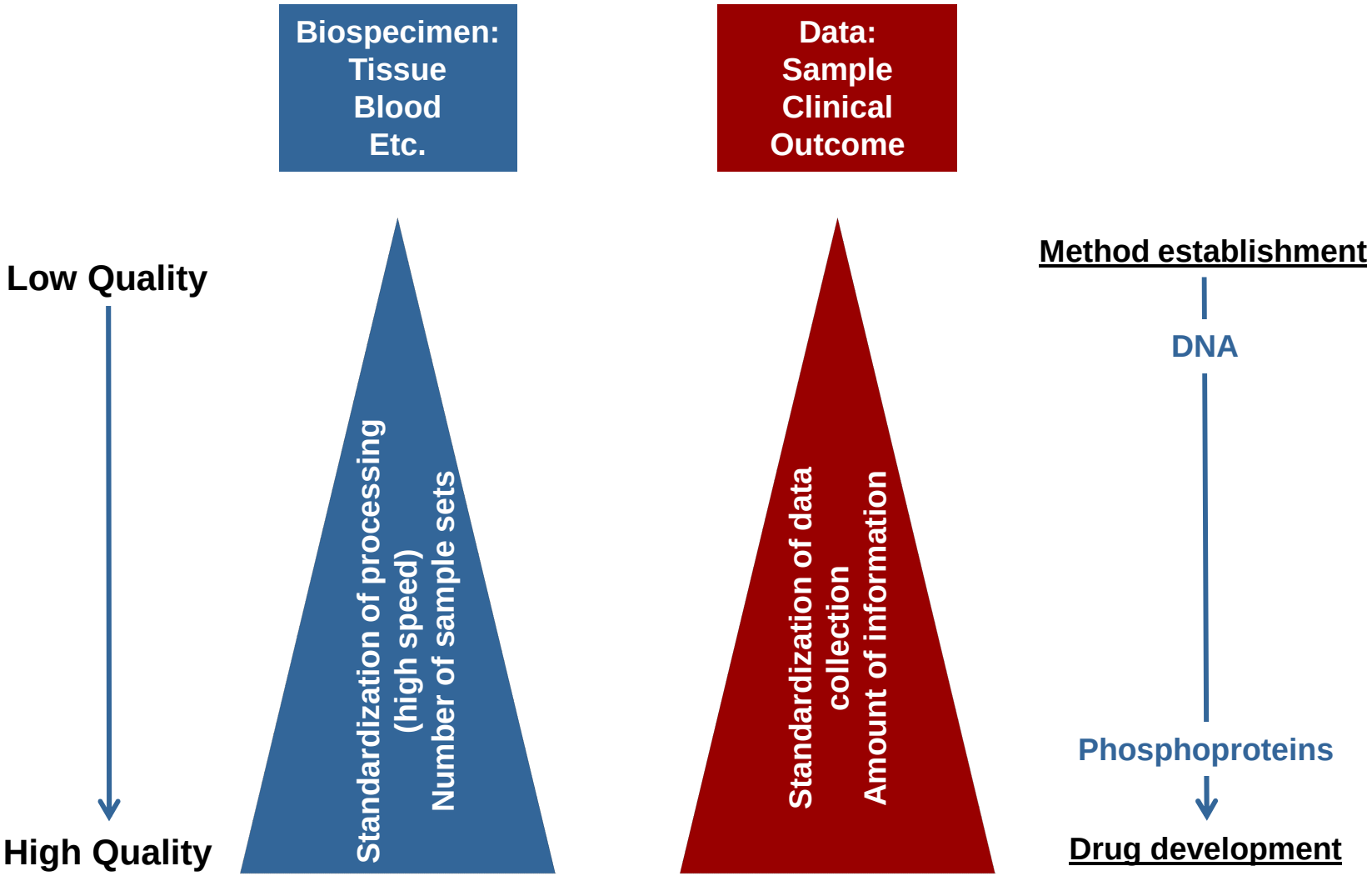
1. Drugs presurgery
2. Surgical steps precollection
3. Warm ischemia time
4. Cold ischemia time
5. Processing
6. Histology Control
7. Storage Conditions

Indivumed Biobank:
The Gold Standard – Providing a Unique Research Tool



- > 17,000 patients
- All major tumor entities
- 22 different normal tissue
- Matched plasma/serum/urine
- > 500.000 samples
- Identical processing of samples
- > 4,5 Mio clinical data points (300 data points/patient)
- Follow-up/outcome information
- ***Tissue processing time ~ 7 min.***

Quality of Tumor-Biobanks



A long way to cure cancer:
Status 1971: Nixon declared „War against Cancer“

A long way to cure cancer:
Status 2013: translation of knowledge to individualized cancer therapies